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THE COMPLEX NATURE OF THE EFFECTS OF TEMPERATURE ON THE RATES OF CERTAIN BIOLOGICAL PROCESSES[1]

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THE rates of most of the processes with which biologists, as well as physicists and chemists, have to deal are profoundly influenced by temperature. The normal fluctuations in what is loosely called "room temperature" may be responsible for differences of several hundred per cent. in the rates of some of these processes. Probably no other single factor causes more difficulties for the physiological worker who wishes to obtain constant and reproducible results than the unavoidable temperature changes which occur during the course of his experiments.

However, this pronounced influence of temperature on physiological processes is not an unmixed evil. From the magnitude of the effect produced by a change of, for example, ten degrees, it is frequently possible to obtain a useful clue as to the fundamental nature of the process in question. Thus, photochemical reactions give values of Q_{10} (the ratio of the rate at one temperature to that at another ten degrees lower) but little greater than unity; processes whose rate is limited by the diffusion of

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dissolved substances values of Q_{10} in the vicinity of 1.25 or 1.3; and chemical reactions of the ordinary sort values which are usually in excess of 2. In the case of the last type of reactions, Crozier has recently attempted in an interesting way to carry the analysis still farther and to distinguish between different classes of chemical reactions by means of the numerical value assumed not by Q_{10} but by the constant μ in the van't Hoff-Arrhenius equation. A value of μ in the vicinity of 11,500 is considered by Crozier to indicate a catalysis by hydroxyl ions, one in the region of 16,700 a certain type of oxidation, etc.

Now the constants Q_{10} and μ are most important tools for the physiologist, but care must be exercised in their determination and use. Under certain conditions the values obtained may be meaningless or even misleading, as will be illustrated by the case about to be described.

Several years ago it became necessary to secure information about what is rather loosely and not entirely correctly called the temperature coefficient of cell permeability. The particular point at issue was whether the effect of temperature was chiefly upon the rate of diffusion of the penetrating substance or whether the cell itself was affected in some more complicated way. It appeared that a value for Q_{10} of 1.25 or 1.3 would indicate the probability of the former, and some very decidedly different value that of the latter alternative. An examination of the rather scanty data in the literature revealed, as might have been expected, figures which were very different for different cells and for different substances. However, even in the rare instances where different workers had studied essentially the same process, there was far from satisfactory agreement as to the value of Q_{10}. It seemed desirable, therefore, to study the question afresh, and for this purpose the case of the entrance of the commonest of all physiological substances, water, into a cell of very simple structure, the mammalian erythrocyte, was chosen.

When an erythrocyte is placed in water, or in a hypotonic solution, it swells. If the solution be sufficiently hypotonic, hemolysis occurs. By the proper choice of conditions, which need not here be discussed, it is possible to secure cases of hemolysis where factors other than osmotic ones are of minor importance and where the disappearance of the erythrocyte represents the attainment of a certain volume, which in turn represents the entrance of a definite quantity of water—usually from fifty or less to possibly one hundred per cent. of the original volume of the cell. The rate of hemolysis may therefore, though with considerable caution, be used as an index of the rate of entrance of water into the erythrocyte.

The ordinary methods for studying the rate of hemolysis are not sufficiently rapid for work such as that here reported where the entire process is usually completed in from one to ten seconds. It was found, however, that by using a simple apparatus, which will be described in detail elsewhere, for measuring changes in the turbidity of a suspension of erythrocytes, the course of even very rapid hemolysis could be followed with considerable accuracy. Since a given reading of the instrument with the same blood always involved the same degree of hemolysis, it was only necessary in order to compare the rates under different conditions to set the instrument in advance at a fixed point and then to determine the various times required to reach this point. The reading chosen for most of the experiments corresponded to approximately 75 per cent. hemolysis of the blood used, which in all of the work here described was that of the ox.

The results of the first series of experiments proved to be rather disappointing. In many cases, Q_{10} values of approximately 1.25 or 1.3 were obtained, but in other instances, for no apparent reason, the values were much larger or much smaller, and, in general, were quite unpredictable. Even worse, at times, not only the magnitude but the direction of the effect was changed and a faster rate was obtained at a lower than at a higher tem-

perature. Though the experiments in their entirety seemed to indicate a diffusion effect, it would have been possible by selecting at random as small a number of cases as are sometimes cited in biological work to have justified almost any conclusion whatever. Actually, the only conclusion that was drawn from these preliminary experiments was that for some unknown reason the experiments themselves were worthless.

The difficulty did not appear to lie either in the apparatus or in the material. The former was shown by appropriate tests to be capable of yielding a very satisfactory degree of accuracy, and the latter, as biological material goes, is known to be remarkably uniform. Evidently there was some theoretical defect in the manner in which the experiments were being carried out which prevented them from yielding results of value. This defect was finally brought to light by considering the underlying theory of the osmotic swelling of cells, and it proved to be a very simple one in principle—so simple, in fact, that it would appear superfluous to mention it here were it not for the fact that it seems to have been so generally disregarded in the past by biologists and physiologists. Stated briefly, it was the confusion of a primary effect on a rate with a secondary effect produced by a change in a position of equilibrium.

An illustration will make this distinction clear. The rate at which a gas diffuses through a membrane depends first and primarily on the specific properties of the gas and of the membrane. The so-called diffusion constant is a measure of these specific properties. Krogh, for example, has found that the diffusion constant for carbon dioxide for certain animal tissues is about thirty-five times as great as that for oxygen. Under the same conditions, therefore, carbon dioxide will diffuse thirty-five times as rapidly as oxygen. But suppose that the partial pressure of carbon dioxide on one side of a membrane is 760 mm., and on the other is 750 mm., while the corresponding figures for oxygen are 760 mm. and zero, re-

spectively. Under these conditions the carbon dioxide which has almost reached its diffusion equilibrium will, in spite of its high diffusion constant, cross the membrane more slowly than the oxygen which is still far removed from its equilibrium.

Now the situation in the case of the erythrocyte is entirely similar. Starting with osmotic inequality we approach by the swelling of the cell osmotic equality. The observed rate of swelling at any given instant should, therefore, depend not merely on the fundamental nature of the water and of the membrane, but on the distance of the system from osmotic equilibrium. Mathematically, we may assume that the rate of volume increase is described by the equation:

$$\frac{dv}{dt} = kA(p - P) \qquad (1)$$

where k is a constant depending on the properties of water and of the cell membrane, A the area of the latter, p the variable osmotic pressure within the cell and P the (fixed) osmotic pressure of the external hypotonic solution. By making certain plausible assumptions as to the nature of A and the relation between p and V we may integrate equation (1), obtaining either

$$k = \frac{V^2 - V_o^2}{2p_oV_oAt} \qquad (2)$$

or

$$k = \frac{p_oV_o}{P^2At} \ln \frac{p_oV_o - PV_o}{p_oV_o - PV} - \frac{V - V_o}{PAt} \qquad (3)$$

according to whether P is, or is not, equal to zero. p_o and V_o in equations (2) and (3) are the initial osmotic pressure and volume of the cell, respectively.

Equations (2) and (3) contain within themselves the complete solution of the difficulty encountered in the preliminary experiments. If we plot the calculated swelling curves for a series of values of the fundamental constant k, keeping P, on which the final equilibrium volume depends, constant, we obtain a condition represented in

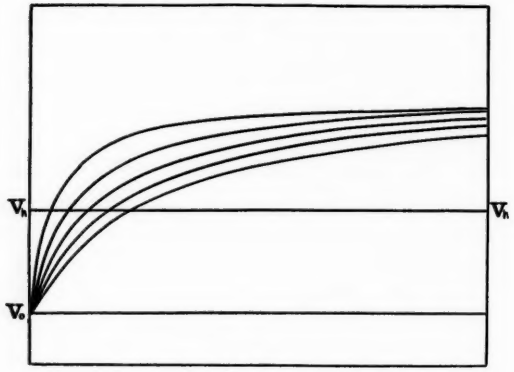

FIG. 1

Figure 1. Starting with the volume V_o the erythrocyte in each case will swell until its volume reaches the hemolytic volume V_h, at which point the cell disappears and the curve comes to an end. The abscissae corresponding to the intersections of the various curves with the line $V_h - V_h$ are the times required for hemolysis in each case and are inversely proportional to the values of the velocity constant k.

In Figure 2 are plotted the curves which result when k is kept constant and P is varied. In the preceding case the final equilibrium is the same and the velocity constant

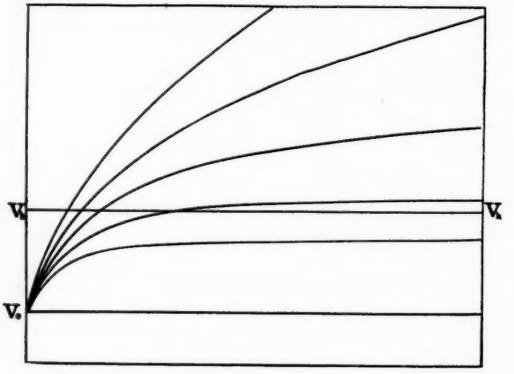

FIG. 2

is different for the various curves; here the velocity constant is the same and the final equilibrium different. As before, the times required for hemolysis are given by the intersections of the curves with the line $V_h - V_h$. It will be noted that there is one rather striking point of difference between the two sets of curves. Small changes in k in the first set are able to produce only small changes in t, the observed time of hemolysis. The same is true for small changes in most values of P in the second set. However, in the case of those values of P which are associated with a theoretical equilibrium volume very close to the line $V_h - V_h$, a small change in P may produce an enormous change in t; in fact, a very moderate change in P may increase the value of t from perhaps a minute or less to infinity by shifting the final equilibrium volume below the critical volume, V_h. For this reason results obtained in this region may be expected to be very untrustworthy, since relatively insignificant variations in the value of the theoretical equilibrium volume can produce enormous variations in the time of hemolysis.

Now it is known that a number of factors, such, for example, as the pH and the oxygen content of the external solution have an effect on the degree of swelling of erythrocytes. Furthermore, the method used requires for complete accuracy that the concentration of erythrocytes must in all of the experiments of a given series be exactly the same—a requirement which it is impossible to meet with entire success. There are, therefore, always present a number of unavoidable sources of error which are enormously exaggerated within a certain limited range of values of the osmotic pressure of the external solution.

Furthermore, in addition to the purely accidental effects of extraneous factors, such as those mentioned, there is an effect on the final equilibrium produced by temperature itself. It has long been known that low temperatures tend to increase the swelling of erythrocytes. This effect, which can easily be studied quantita-

tively by the method used in these experiments, is probably an osmotic one produced by a change in the distribution within the cells of base between hemoglobin and other acids. In any event, it is always present and is of such a nature as to act in the opposite direction from that of the primary effect of temperature on the velocity constant itself. A rise of temperature tends to increase the value of k, and in this way the rate of hemolysis. But at the same time, by its effect on the theoretical equilibrium volume, it also tends to oppose hemolysis. In the cases where a faster rate is obtained at the lower of two temperatures the latter opposing effect actually predominates. In general, in the region in question the two effects are so entangled with one another and with extraneous sources of error that the actual results are frequently unpredictable both as to magnitude and direction.

Now in the experiments mentioned above, it happened, unfortunately, though rather naturally, that the osmotic pressure of the external solution was such as to bring the equilibrium volume very close to the critical volume, V_h. Before the theoretical aspects of the situation had been sufficiently considered it was thought that the greatest accuracy could be obtained when the times to be measured were fairly long, and solutions just sufficiently hypotonic to cause hemolysis were therefore purposely chosen. After the source of the difficulty had been discovered and a change had been made to more dilute solutions, the indirect effects on rate produced by simultaneous changes in the equilibrium volume immediately became insignificant and perfectly consistent results could be obtained at will.

The results of these later experiments will be published elsewhere by the author and Miss Mary Morrison, but it may be stated here that for a range of temperature from 0° C. to 40° C. the values of Q_{10} obtained for water alone, for dilute solutions of NaCl and for dilute solutions of sucrose are all in excellent agreement, the average values

in every case lying very close to 1.3. This figure is slightly reduced if allowance is made for the effect of temperature on osmotic pressure itself, but in either case it lies within the range of values believed to be typical of diffusion processes.

It may be mentioned further that although the value of the constant k at a given temperature is very different for solutions of electrolytes such as NaCl and of non-electrolytes such as sucrose, the temperature effect is almost the same in both cases, and is identical with that obtained with water alone. It would appear, therefore, that as far as conclusions may be drawn from experiments of this sort, the intake of water by the erythrocyte of the ox is a relatively simple diffusion process or at least such a diffusion process exerts a controlling influence upon the rate of the process as a whole.

These experiments have been presented at "The Symposium on Temperature Effects," not so much for their own sake as to serve as an illustration of the danger of confusing rate and equilibrium factors in temperature studies. This is not infrequently done. One case in point has to do with the phenomenon of adsorption. The *amount* of material held by a given adsorbent is increased by lowering the temperature. But the fundamental rate at which the adsorption equilibrium is approached tends under the same condition to be slowed. The situation is, therefore, analogous to the one just discussed, and it is evidently not permissible from temperature coefficients alone to conclude, as has frequently been done in the past, that a given process is, or is not, one of adsorption. Other similar instances would not be difficult to find.

In conclusion, it is suggested that in quantitative studies on temperature effects care should always be used to determine whether an equilibrium as well as a rate effect is conceivably involved and, if so, that the experiments should be carried out in such a manner that the two factors can, as far as possible, be separated.

TEMPERATURE AS AN ECOLOGICAL FACTOR IN ANIMALS[1]

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IT would be highly presumptuous to attempt to recite all the possible effects of temperature in the ecology of animals, for relatively little is known about the subject. Nor would it come within the legitimate scope of this symposium to attempt to recite all of the investigations with regard to the subject. Rather than to produce the dulling effects of prolonged and comprehensive citation of details, important though they may be, an attempt will be made in the course of a brief survey of this field to cite certain outstanding features of research which have contributed much to our recent knowledge of the subject and promise much in the future, in the hope that these may stimulate the production of new ideas and the initiation of new research projects.

For the purpose of the present discussion the subject of "Temperature as an Ecological Factor in Animals" will be arbitrarily divided under the heading, first of "Temperature as a Factor in Animal Distribution" and secondly, "Temperature as a Factor in the Equilibrium of Natural Animal Communities." The subject of the text upon animal distribution will be divided, first into distribution in space, and, secondly, into distribution in time. The subject of distribution in space will be divided first into geographic distribution and, secondly, local distribution. Under the subject of geographic distribution we shall consider, first, the effect of temperature during

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the period of activity and, secondly, the effect of temperature during the period of dormancy. The subject of the distribution in time will be divided into diurnal activity and seasonal activity. The discussion will now proceed as outlined.

To simplify matters in this short consideration, the subject will be restricted to poikilothermic animals. The homoiothermic, or warm-blooded animals, carry their temperature environment with them, consequently the subject is quite different from that of the cold-blooded animals, which are more directly subject to temperature changes.

The effect of temperature upon the geographic distribution of animals has been a subject of discussion since the time of the earliest investigations of animals. Since the temperature was one of the most obvious factors which varied more or less directly with the distance from the equator, various maps have been constructed, zones of animal life have been drawn, and even so-called laws have been formulated as to the effect of temperature in governing animal distribution. These facts are so well known that a knowledge of them may be assumed for the present discussion.

During the period of activity it is classically known that development begins with the ascent of temperature in the spring and ends with the decline of temperature in the fall. However, the situation is not as simple as that, and Roubaud (1922) has recognized homodynamic forms which are able to pass through generation after generation so long as the temperature is favorable and heterodynamic forms which pass through a stipulated number of generations and then stop regardless of temperatures. In general, the stopping is synchronized with temperature cycles, but it is not always so. Complications of this sort are seen in the silkworm, where Jucci (1926) designates the univoltine and bivoltine races, depending upon whether they pass through one or two successive generations without dormancy. This matter has assumed some

importance in connection with the European corn borer (*Pyrausta nubialis*).

The question at issue in this case is whether the effect of temperature will change the genetic constitution of the organisms so that what was a one-generation race may become a two-generation race, or whether temperature acts upon a heterozygous culture of animals and selects out of a certain environment the one-generation race and out of another environment the two-generation race. It must be clearly recognized that if the first is true, that is, that temperature acting over a period of time will cause a race of one- or two-generation animals to develop and inherit a fixed number of generations per year, we then have a case of the inheritance of an acquired character. The evidence to support the effect of temperature in producing a one- or two-generation race as an inherited character must be just as good as the evidence required to prove the entire case of the inheritance of an acquired character.

In the case of the promethea moth, there are two generations in the southern part of the United States and one generation in the north. In the event that the moth were to complete one generation and the entire population in the northern states were to pass into the second generation, but not be able to complete development to the point of pupation they would all be killed in the winter time because they are unable to endure dormancy in any other state than as pupae. If the ability to pass through one or two generations per year constitutes a genetic character, why are there not one-generation forms in the south as well as the two-generation forms? The unpublished work of Dawson shows that the southern two-generation race can be forced to pass through but one generation a year and that the northern one-generation race can be forced to pass through two generations a year. However, the tendency for one generation and the tendency for two generations seems to be influenced by a succession of changing temperatures, such that we

have in nature the tendency for one and the tendency for two generations a year, the temperature acting as a controlling factor in determining which of these tendencies is to be suppressed. The matter of dormancy after the spinning of the cocoon is determined early in larval development and subsequent exposure to low temperature will not stop development. If the temperature is high enough to permit development at all transformation will take place and the adults will emerge.

The effect of temperature during the period of dormancy is of great biological importance and of absorbing interest in the field of research. Not all poikilothermic organisms undergo a period of dormancy. Some migrate to avoid low temperatures, either over geographic areas or make local migrations to avoid low temperature. In the present brief discussion dormancy at low temperature alone will be considered. Rasmussen (1916) and Polimonti (1912) have made summaries of the various theories of hibernation. Rather than to delve into these, attention will be called to certain recent researches which seem to throw new light upon this very old subject.

Nellie Payne (1926) showed that certain wood-boring beetle larvae which are exposed under the bark of trees throughout the winter change their temperature relationships during the fall. Due to a loss of their free water their freezing points and under-cooling points undergo a gradual lowering as the low temperature comes on. Consequently they are able to endure very low temperatures during the winter without freezing and, furthermore, they seem to be able to withstand freezing when in this condition. As spring comes on they take on more free water and their freezing points rise again. It was possible experimentally to lower the freezing point by desiccation during the summer and to raise it by the addition of moisture during the winter.

Robinson (1927) has made a study of three insects, the promethea moth which habitually hibernates in an exposed cocoon attached to the twigs of trees, the larvae of

certain lamellicorn beetles which are called white grubs, which habitually hibernate in the soil and migrate downward in the fall as the frost line goes down, and the supposedly tropical granary weevil, *Sitophilus granarius,* which is unable to endure dormancy at all. By an ingenious method Robinson has been able to distinguish between the water which exists in the body of the insect as free water and that which exists as colloidal water bound upon the surfaces of the colloids. With the promethea moth a series of determinations coincident with a gradual lowering of temperature has shown that the amount of free water gradually diminished and the amount of bound water gradually increased as the temperature went down. This is consistent with the work of Newton and Gortner (1922) on wheat. During a period of fifty days it was found that the bound water increased from 52 to 320 milligrams and during the same period the free water dropped from between six and seven hundred milligrams to between three and four hundred milligrams.

Similar observations upon the lamellicorn beetle larvae, which habitually migrate down into the soil to avoid the lowest temperatures, show that the total water content remained about constant and that there was a very slight gain in colloidal water. Ten weeks of exposure to a temperature slightly above that at which they would freeze resulted in a change of bound water from only about eighty milligrams to 110 milligrams, or an increase of about two per cent. In the case of the granary weevil, in similar experiments, there was a gradual loss in total water, a gradual decrease in the amount of bound water and a gradual decrease in the amount of free water which eventually resulted in the death of the beetles. Thus, in the case of these three species, the promethea moths are able to endure very low temperatures and they have a large per cent. of their water stored as bound water. The more or less indifferent beetle larvae have only a small increase in bound water as the

winter goes on. The granary weevil, which can not resist low temperature, loses its water and has actually a decrease in bound water. A species which is able to change its water from free to bound condition as low temperature comes on undergoes a process of hardening or increased resistance to low temperature. The freezing point of the bound water is lowered and the bound water will be conserved during the long period of dormancy.

Any given species, when exposed normally to low temperature, will probably react in a regular and definite manner according to the specific characteristics of its hydrophilic colloids. Since these are known to vary greatly in different organisms it may be that species have very definite inherent limitations beyond which they can not go because of their inability to endure low temperature or to prepare themselves for it by the so-called process of hardening. In so far as geographic distribution is influenced by the low temperature of winter, the nature of the hydrophilic colloids may be of primary importance. Robinson's study of a series of insects has shown a direct correlation between the per cent. of bound water and the ability to endure low temperature. This is a significant line of work which will bear further intensive investigation.

The subject of local distribution with respect to temperature has been subject to much descriptive work but regrettably little quantitative investigation. Graham (1920) has shown that environments as small as logs may have zones of widely different temperature, due to the angle of incidence of the sun's rays, which naturally follow a regular daily cycle.

Buxton (1923), Chapman, Mickel, Parker and others (1926) have shown that the surface temperatures of soil often rise far above the temperature which can be endured by the organisms which normally live in the environment. Thus certain bembicid wasps, which are among the most characteristic organisms found on the sand dunes, are confined within an extremely narrow zone of

temperature, between the low temperature at which they become dormant and the high temperature at which they suffer heat paralysis. It was found that nearly every day during the season the temperature on the surface of the sand would exceed the temperature which they could endure for more than a few seconds of time and that every night the temperature of the sand dropped below the temperatures at which they could be active. During the heat of the day these wasps were compelled to dig their burrows under the most trying circumstances. After working furiously for a few seconds at a time they would then rise to a height of ten or twelve inches where they experienced a drop of some 15 to 23 degrees centigrade and thus were able to cool their bodies. By repeating such efforts they eventually got a hole sunk below the surface of the sand where the temperature was again within their zone of activity for a prolonged period of time. In a similar way it was found that the activity of many of these insects living upon the sand dunes consisted of a matter of juggling time and space in order that at any particular time they might find a place where the temperature was suitable and in any particular place they could find a time during the temperature cycle of the day in which they could be active. Thus there was one population of animals for the day and another population of animals for the night and the order of their activities throughout the day was determined very largely by the procession of temperature.

We have now come very logically to a consideration of the distribution of organism in time as affected by temperature and it has just been stated that the diurnal activity of poikilothermic organisms may be determined, at least in certain cases, by the procession of temperatures in the specific locations where the organisms may be.

The seasonal distribution of organisms is largely dependent upon the effect of temperature upon the rate or the velocity of development of the organism. This brings

us to a very comprehensive field, the understanding of which rests fundamentally upon a knowledge of the physiology of the animals, and in this case ecology comes into the field of general physiology, just as general physiology, in a search for the explanation of the physiological phenomena, comes very naturally into the field of physics. The fact that the ecologist is in good company when he finds himself on common grounds of investigation with the physiologist, or that the physiologist finds himself in common ground with the physicist in his investigations, is not to be denied regardless of whether the converse is considered to be true.

Various authors tell us that calendars charting the sequence of periodic phenomena were made centuries before the time of Christ. Empirical methods have been employed to make deductions from the records of temperature and the appearance of animals and also from experiments under laboratory conditions with a view to determining and valuating the effect of temperature during the spring of the year upon the appearance of animals. Among the recent attempts to refine these methods we have the publications of Shelford (1927) on the codling moth.

Cook (1927) has recently made a study of the effect of alternating temperatures upon the metabolism and rate of growth of the larvae of certain noctuid moths, which is similar to the much earlier work of Von Oettingen and others. It was found that immediately following a change of temperature, the rate of metabolism was higher and that later it dropped off. The point for most rapid metabolism in one of the species considered by Cook was about 32° C. and the time for most rapid growth per hour is an exposure of about eight hours at 32° C. Such combinations of time and temperature are very common in the natural geographic environment of this species. Thus, by studying the various combinations of time, temperature and metabolism, Cook mapped out a three-dimension surface from which the rate of metabolism or

growth for any time temperature combination might be predicted. Thus the prediction of the seasonal appearance and development of a given species is extremely complicated because of all the possible combinations of time, temperature and metabolism which may be involved. Since the complex nature of the effect of temperature on the rates of biological processes has been considered in another paper it will not be necessary to do more than make these passing remarks on the subject.

Calling attention now to the effect of temperature upon the equilibrium of natural communities of animals, it is necessary to remember first of all that here the ecologist has to consider the effect of temperature upon the rates of development and upon the various functions in a heterogeneous system of various interrelated animals, just as a physiologist has to consider a heterogeneous polyphasic system of physical phenomena and the effect upon each particular phenomenon as well as the algebraic sum of the effects on the entire system as expressed in the rate of metabolism. Since the best quantitative expression of the condition of such a natural community of animals is the number of each species present, it will be well, first of all, to turn attention, very briefly, to the effect of temperature upon the growth of a population of animals.

When the potential rate of increase of a population is compared for two temperatures ten degrees apart, it is found that the principle of van't Hoff and Arrhenius, granted that it might hold for such biological processes as metabolism, does not give us an adequate insight into the rate of increase of a population. Even though it may be true that the number of young produced per unit of time is doubled by an increase of 10° C., it will also be true that the rate of attainment of maturity is halved by an increase of 10° C. Thus we find that, when the number of young is doubled in a given period of time, due to the rate of reproduction, the number attaining maturity in the same period of time is also increased because, at

the higher temperature, there will be two generations in the same period of time that there is one generation at the lower temperature. For example, if we were to assume that a certain species produced ten young per day at 20° C. and twenty young per day at 30° C., and that at 30° C. the young would attain maturity in ten days, we would then assume that they would attain maturity in twenty days at 20° C. Granting, for the sake of this example, that the twenty individuals which attain maturity on the tenth day at 30° C. each give rise to twenty new individuals and that these in turn attain their maturity on the twentieth day, there would then be present on the twentieth day 400 mature individuals, counting only the individuals born on these two days. The ten offspring produced on the first day at 20° C. will attain maturity on the twentieth day and there will then be ten mature individuals at 20° C. and 400 at 30° C.

Thus the discrepancies for a small interval of temperature as expressed in the theoretical number of individuals in a population are so great that it seems possible that the effect of a single degree in the difference of temperature may be measurable in this way.

Wadley's unpublished data show that the curves representing the effect of temperature upon the population increase, individual development and the rate of reproduction per day in *Toxoptera graminum* represent three curves of similar shape but of different slope. In the experiments with these aphids it has been possible to produce curves of actual population increase which are practically identical with the theoretical rate of increase of the population at the different temperatures, so long as other factors are held constant. This seems to suggest the possibility of measuring the effect of factors including temperature in terms of the reduction in the potential rate of increase.

After consideration of the effect of temperature upon the population increase of a single species, probably the next logical step in the building up of a natural commu-

nity is to consider two ecologically related species such as a host and its parasite. On this subject we have the classical example given by Webster and Phillips (1912) of the green aphid (*Toxoptera graminum*) which is able to reproduce at a temperature of about 40° F. and its parasite (*Aphidus testaceipes*) which does not begin to reproduce until the temperature reaches about 56° F. Obviously in an environment, with the temperature ranging between 40° and 56° F., the host population will increase while that of the parasite will remain stationary. However, when temperatures are higher and the parasite begins to reproduce, we have the effect of a parasite on a host population. In this connection it is suggestive to remember that the cases of practical control of insect pests by parasites are practically all of them found in the tropical or subtropical regions where temperature is relatively constant as compared with "temperate" climates. It is obviously impossible to have effective parasitic control unless the host and parasite relationships are such that they are synchronized as to the effect of temperature on their potential rate of development.

Unpublished data are at hand which tend to show that two closely related and competing species of beetles in controlled environments are present in varying proportions, the ratios of the two species to each other being dependent upon their curves of population increase. The species with the higher potential rate of increase at high temperature is dominant at high temperature, while the species with the relatively lower potential rate of increase at the high temperature is present in smaller numbers. The reverse is true at the lower temperatures, however, so that one species is dominant at high temperature and the other species dominant at low temperature, the differences apparently being due to the relative potential rate of population increase at the two temperatures.

It may be hoped that the day is drawing near when the ecologist will be attracted by the possibility of analyzing the relationships involved in the complex natural commu-

nities of animals in much the same way that the physiologist is analyzing the different phenomena concerned in the rate of development of organisms which consist of the complex physical systems. The ecologist will then no longer be tempted to call attention to phenomena which he thinks interesting because they seem to be inexplainable and will center his attention upon the explainable laws which govern the relationships involved in systems as complex and intricate as those between animals in natural communities.

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THE RELATION OF TEMPERATURE TO THE DEVELOPMENT OF DISEASE IN PLANTS[1]

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THE plant pathologist knows the parasite to be the primary cause of disease, but the progress of investigation is defining more clearly each year the fact that in practically all cases the serious development of the disease is conditioned upon certain environmental factors. The explanation of the occurrence and severity of a parasitic disease, the nature of its development and its control requires, therefore, not only the recognition of the "causal factor," the parasite, but also the definition of the "conditioning factors," the environment.[2] Temperature probably becomes one of the predominating conditioning factors to consider in the development of disease in cultivated plants due chiefly to the wide longitudinal

[1] Paper read as a part of the Symposium on "Temperature and Life" at the meeting of the American Society of Naturalists held in Nashville, Tennessee, December 30, 1927. The investigations upon which this paper is based were conducted cooperatively between the Wisconsin Agricultural Experiment Station, Office of Cereal Crops and Diseases, United States Department of Agriculture and Funk Brothers Seed Company.

[2] Credit is given Jones, Johnson, and Dickson 1926[1] for the use of the terms "causal factor" and "conditioning factors" to denote the parasite and environmental conditions respectively in terms of their influence upon disease development.

distribution of plants for agricultural purposes.[3] In other words, the problem of adaptation of agricultural plants to widely different environmental conditions has made a study of the "conditioning factors" in relation to host and parasite and their intimate relationship the first essential in understanding the development of the disease and in selecting for disease resistance. Such a study of the temperature complex, for example, soon offers the opportunity of selecting individuals from the so-called varieties which will develop successfully under the unfavorable environment. The use of scientific methods in the propagation, selection and breeding of the individuals to speed up natural selection leads to the development of disease-resistant lines as a control for the disease by a tolerance of the unfavorable conditions. Expressed in different phraseology, which indicates more nearly the time involved, it is the stabilization of new plant strains resistant to the unfavorable conditions. These new lines can then be studied in detail by the plant geneticist, the plant physiologist, the plant chemist and the cytologist to explain the nature of this resistance. Because the morphology and physiology of the inbred lines becomes more uniform as selection continues, and because series after series of the plants can be grown under the same or varied environments at the experimenter's will, temperature as a factor of the environmental complex then becomes a tool for the selection, development, and study of new improved varieties of plants.

This paper will be restricted to the presentation of certain examples of the influence of temperature upon the development of disease in plants. It is evident, of course,

[3] Brinton 1868[2], Hoy 1883[3] and Stickney 1897[4] all have remarked that the Indians' development of the semi-tropical plant, corn, into a great food supply so far north as it was found forms the subject of an interesting study of the agricultural instincts of the Indian and that while the plant may have moved east or west accidentally, "skilful cultivation and attention to the selection and improvement of variety were necessary to its northward movement."

that other variables in this complex may play an even more important part in certain instances than does temperature. This is obviously true of moisture and soil fertility and may hold conceivably for variations in soil reaction, aeration, light or any factor or factor-complex which materially affects or modifies the plant's nutrition or other metabolic processes. The mere listing of these indicates that variation in more than one important factor must be occurring simultaneously. It seemed wise, however, to vary one factor at a time in studying the influence of the environmental complex, keeping the others as nearly optimum for the given set of conditions as possible. While the results presented in this paper, therefore, deal largely with the influence of temperature, the effect of the environment as a whole upon the plant has been constantly in mind. The controlled experiments have always been checked and correlated with field experiments in different localities to further guard against limited interpretation of results.

Temperature influences the development of the plant disease when under the existing set of conditions it modifies the functional processes of host or parasite sufficiently to change their intimate interrelationship. That is to say, a certain modification of range of variation in one or more factors of the environmental complex changes the existing plane of metabolic balance and sets into action new physiological processes which may make the parasite incapable of further aggressive development on the one hand or the host resistant to invasion on the other. With this conception of the problem in mind, the discussion will be directed, first, toward the influence of temperature upon the development of certain diseases; second, upon the use of this information in selecting resistant lines, and finally, third, upon the nature of resistance to disease in these special instances.

TEMPERATURE INFLUENCES THE DEVELOPMENT OF SEEDLING BLIGHT OF WHEAT AND CORN

A seedling blight of the cereal crops, especially of wheat and corn, occurs in the Central and Eastern portion of the United States and causes local or general damage each year, depending largely upon environmental conditions. The parasites, principally *Gibberella saubinetii* (Mont.) Sacc., are seed-borne as well as soil-borne and cause heavy losses, especially where corn or wheat are in close sequence in the crop rotation.[5] This parasite invades the cortical tissues of the young seedlings, causing a rot which kills the plants near the time of emergence.

Before discussing the influence of temperature upon the development of this seedling blight of wheat and corn caused by the fusarial stage of *Gibberella saubinetii,* it seems advisable to compare briefly the relation of temperature to the two host plants.[6] Wheat germinates and grows during the early seedling stage at temperatures ranging from about 1° to 36° C. depending somewhat upon the variety and condition of the seed. The rate of growth increases with the rise in temperature to about 28° C., after which it falls off rapidly. The stocky, well-developed seedlings with a large root system occur at comparatively low temperatures, 4–12° C., depending here especially upon the variety grown. Corn germinates and grows in the seedling stage at temperatures between 6° and 40° C. The rate of growth increases with the rise in temperature to about 32° C. The well-developed seedlings with a good root system occur at temperatures around 20–24° C., or 12° to 16° C. higher than the wheat seedlings. The young wheat seedling, therefore, is essentially a low temperature plant. The corn seedling, on the other hand, is a high temperature plant. See Figures 1 and 2.

The parasite producing the seedling blight grows best at high temperatures. Although the spores will germinate and the mycelium grow at temperatures from 4° to

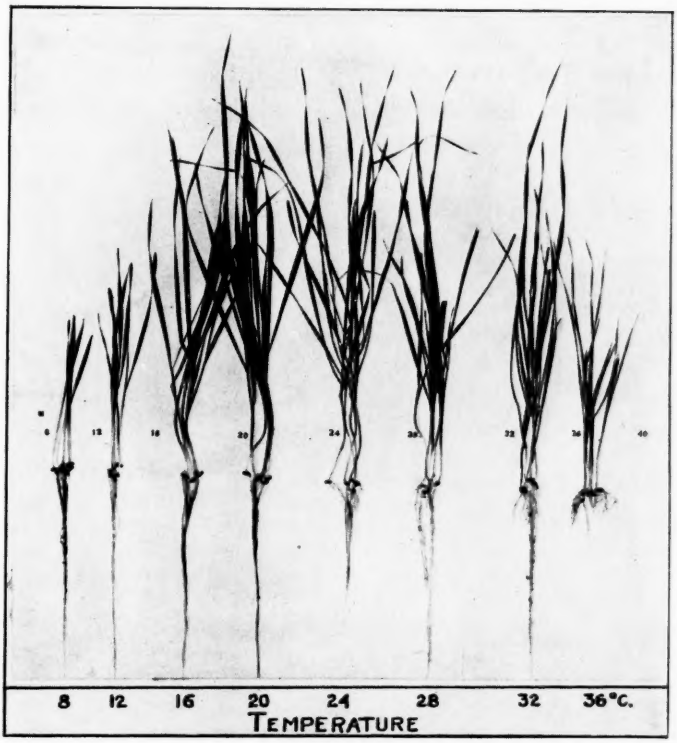

FIG. 1.—THE RELATION OF TEMPERATURE TO THE DEVELOPMENT OF MARQUIS WHEAT SEEDLINGS

PLANTS GROWN TWENTY DAYS AT THE TEMPERATURES INDICATED TO THE LEFT OF EACH GROUP OF SEEDLINGS. NOTE THE LARGER PLANTS AND GREATER ROOT DEVELOPMENT AT THE MEDIUM LOW TEMPERATURES.

32° C., yet the most aggressive development occurs at 24 to 28° C., depending upon other conditions of the environmental complex.

THE TEMPERATURE INFLUENCE ON THE DISEASE IS DIFFERENT WITH TWO HOSTS

The wheat seedlings blight at high temperatures and the corn seedlings at low temperatures. Wheat seed-

lings, low temperature plants, blight at temperatures of 12° C. and above and are resistant at temperatures around 8° C. Corn seedlings, high temperature plants, blight at temperatures below 20–24° C. and are resistant

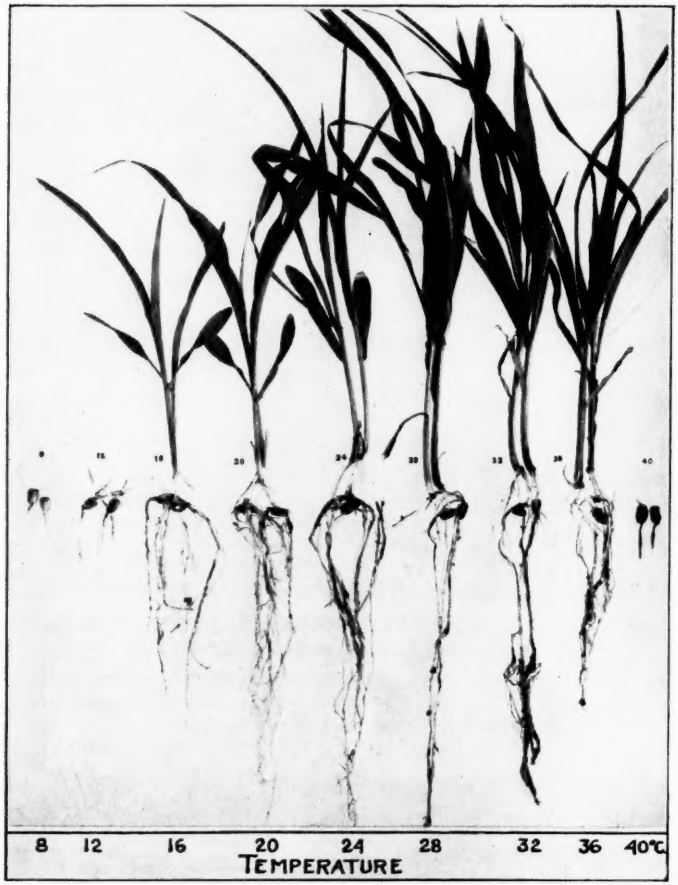

FIG. 2.—THE RELATION OF TEMPERATURE TO THE DEVELOPMENT OF AN INBRED LINE OF CORN, B–R–10–12

PLANTS GROWN TWENTY DAYS AT THE TEMPERATURES INDICATED TO THE LEFT OF EACH GROUP OF SEEDLINGS. NOTE THE LARGER PLANTS AND GREATER ROOT DEVELOPMENT AT THE MEDIUM HIGH TEMPERATURES.

above 24° C. (See Figures 3 and 4.) Is this difference in response due primarily to the influence of temperature upon the host plants or upon the parasite? If upon the parasite, the blight should be more severe at the relatively high temperatures, which favor the growth of the fungus. This condition is well illustrated in the relation of temperature to the wilt diseases, which will be dis-

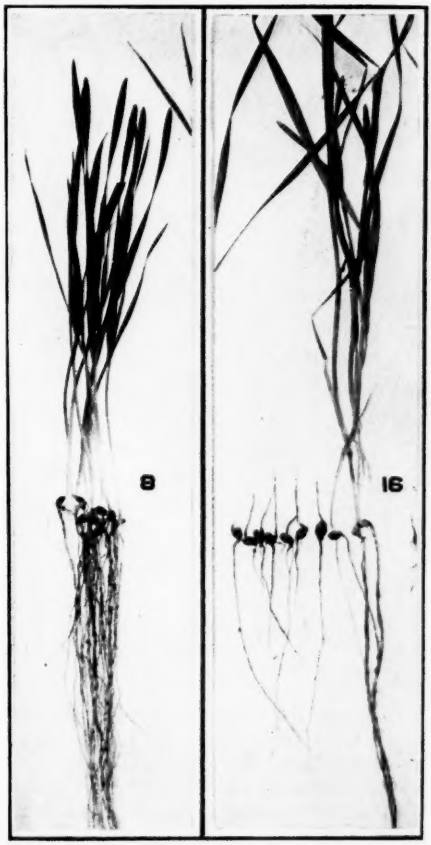

FIG. 3.—WHEAT SEEDLINGS BLIGHT IN A WARM SOIL

THE SEEDLINGS GROWING IN A "WHEAT SICK" SOIL BLIGHTED BADLY AT 16° C. IN CONTRAST, THE PLANTS GROWN AT 8° C. WERE HEALTHY EVEN THOUGH THE SEEDLING BLIGHT PARASITE WAS PRESENT IN THE SOIL.

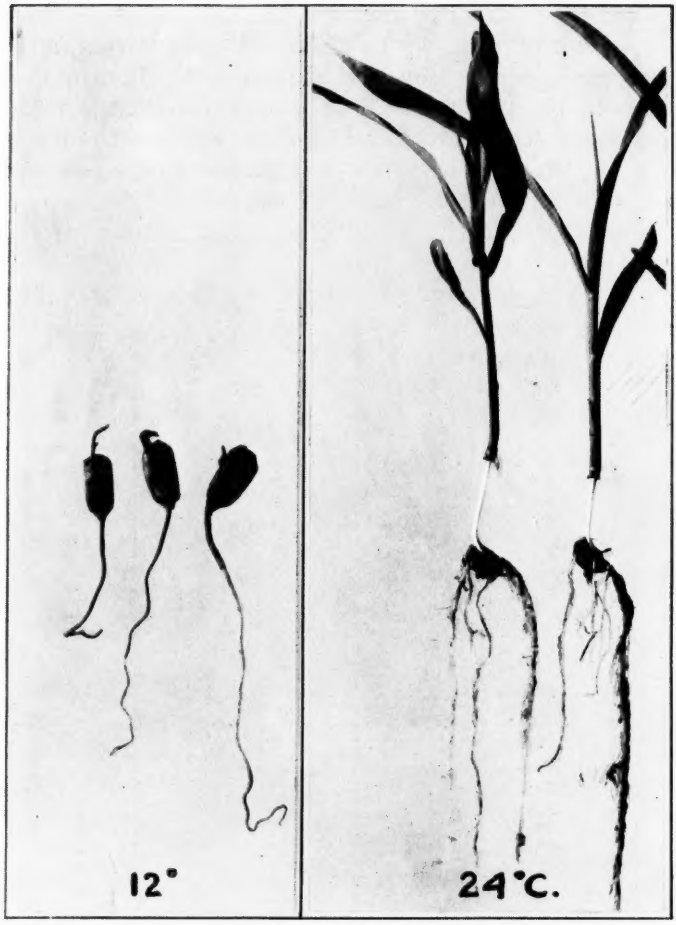

FIG. 4.—CORN SEEDLINGS BLIGHT IN A COLD SOIL

THE SEEDLINGS GROWING IN A COLD SOIL, 12° C., ARE KILLED BY THE SEEDLING BLIGHT PARASITE BEFORE THEY EMERGE, WHEREAS THOSE GROWING AT 24° C. REMAIN HEALTHY IN A SOIL HEAVILY INFESTED WITH THE SEEDLING BLIGHT PARASITE.

cussed in the next chapter. If upon the host plants primarily, the blight should be different with the two hosts because their temperature response is different. This would indicate that disease development in this instance

was closely associated with the physiological functioning of the wheat and corn seedlings at different temperatures. Here then is a challenge to use environment, temperature in particular, as an instrument to study the cause of disease development and the nature of disease resistance.

Temperature Influences the Development of the Wilt Diseases in Plants

A wilt disease is a definite yellowing and wilting due to the activities of a specific fungus within the vascular system of the plant. In these respects it is unlike the cortical rots or blights, where one parasite may invade the outer, soft or parenchymatous tissue of a wide range of plants and ultimately kill the plant by an insidious development of this soft rot. The flax wilt, cabbage yellows, cotton wilt and tomato wilt are examples of this type of disease. In all these wilt diseases the plants are free from wilt at comparatively low temperatures. Each plant species has a fairly definite temperature limit below which wilt does not occur. This so-called lower critical temperature for wilt development varies somewhat with the different species, being about 14° for flax wilt,[7] 15° for cabbage yellows,[8] 20° for cotton wilt,[9] and 20° C. for tomato wilt.[10] It is interesting that here again there are indications of the minor influence of host response to temperature in that cotton and the tomato, both being high temperature plants, have a minimum for wilt development about 5° C. higher than the low temperature plants. In general, however, the temperature curve for wilt development corresponds very closely with the temperature curve for growth of the respective specific parasites. And these curves, in turn, are very similar to the temperature curve of *Gibberella saubinetii*, the seedling blight organism, thus indicating that all of these organisms are high-temperature fungi whether they are high-temperature parasites or not. These differences in parasitism may be answered more clearly in a later chap-

ter on the development of disease and the nature of resistance.

The temperature reactions of a sufficient number of diseases have been given to show the reader that each specific disease will develop aggressively at certain temperatures, whereas at other temperatures it does not develop. It is possible then under controlled greenhouse conditions, or in the field, to produce the disease in severest form by modifying temperature in the greenhouse or date of planting in the field. In all probability, other factors of the environmental complex can be modified in the same way, although not so conveniently, with similar results. This means, therefore, that with most diseases an environment can be made favorable for the uniform development of the disease and that varieties or strains of plants can be tested for resistance to disease under these so-called optimum conditions. As a result, temperature control becomes a tool with which the selection for resistance, based on the principle of the survival of the fittest, can be carried on indefinitely.

TEMPERATURE AIDS IN SELECTING FOR DISEASE RESISTANCE IN PLANTS

The temperature at which disease develops was found to vary with individuals or groups of individuals. This is not surprising for variations in function occur over wider ranges than even variations in structure of plants. Here then was the challenge to the plant pathologist—the opportunity to select for disease tolerance or even disease resistance.

The earliest work in which the unfavorable environment was used explicitly to select resistant individual plants was done by L. R. Jones and associates[11] in selecting for resistance to cabbage yellows. This early work was field selection where heavy infestation with the parasite and high soil temperatures made the resistant plants stand out distinctly from the mass. Bolley[12] and Orton[13] were probably the first to make field selections for resis-

tance to flax wilt and resistance to cotton wilt, cow-pea wilt, etc., respectively, but with little specific knowledge of the influence of the environment upon the expression of this resistance. Practically all of this selection, except the more recent work with cabbage and flax, has been done in the field with no attempt being made to use the controlled environment as a means of hastening and continuing selection.

Controlled environment, as well as field conditions, have been used in selecting corn strains resistant to seedling blight and root rot. The inbred lines are first "indexed" in the control greenhouses, under low temperature conditions, in disease-infested soil for resistance to seedling blight and root rot as well as cold resistance in the seedling stage. By this method, it is possible to select strains in the segregating inbred lines which are highly resistant to these diseases and which will also grow at very low temperatures. For example, the seedlings of the parent stock of yellow dent corn from which several of the best, resistant lines have been obtained will not form chlorophyll below 16° C. Lines have been selected which will form chlorophyll and grow as low as 8° C. Much of the selecting and stabilizing for factors dealing with function independent of morphological characters must be done under controlled conditions where the testing can be done repeatedly under the same environmental conditions with large numbers from each generation. In fact, much of the detailed study of plant physiological reactions can best be made under controlled environments where large numbers of individuals as well as frequent replications can be grown with precision and accuracy. After this, these experiments must be checked with field plantings where fluctuating conditions exist before final interpretation of results can be made or conclusions can be drawn.

The complex of biological reactions called disease is the resultant of the interaction of a more or less plastic host and a plastic parasite under the influence of a vary-

ing environment. The problems, approached with this conception, involve highly complex phases of plant physiology whether dealing with problems of general metabolism of host or parasite or with the intimate cellular reactions between them.

TEMPERATURE AND THE NATURE OF RESISTANCE TO DISEASE IN PLANTS

The marked difference in the influence of temperature upon the development of the seedling blight of wheat and corn (Figures 3 and 4) was an immediate challenge to study the nature of this response and its relation to the expression of resistance to seedling blight. The wheat seedling blighted at temperatures above 10° to 12° C. and remained healthy at temperatures below 10° C. In contrast, the corn seedling blighted at temperatures below 24° C. and remained healthy at temperatures above 24° C. This makes a difference of 16° C. or thereabouts between the temperatures which enabled the seedlings of the two species to develop healthy tissues in the presence of the parasitic fungus. The temperatures at which these naturally resistant seedlings developed were also the most favorable temperatures for the development of sturdy, tough, plants. The first point to be investigated, therefore, was the intimate cellular relationship between the hosts and their common parasite.

Cell wall penetration different

The cell walls of the cortical sheath tissues checked invasion at temperatures where the seedling plants remained healthy. In the formation and growth of the seedling roots of these plants, the root originates from the central stele inside of the protective sheath tissue, of the coleorhiza, and grows outward through this tissue to the surface. This is the first mechanical break in the sheath tissues surrounding the seedling. The surface layer or epidermis of this root sheath is resistant to fungus invasion. See Figures 5 and 6. In the early

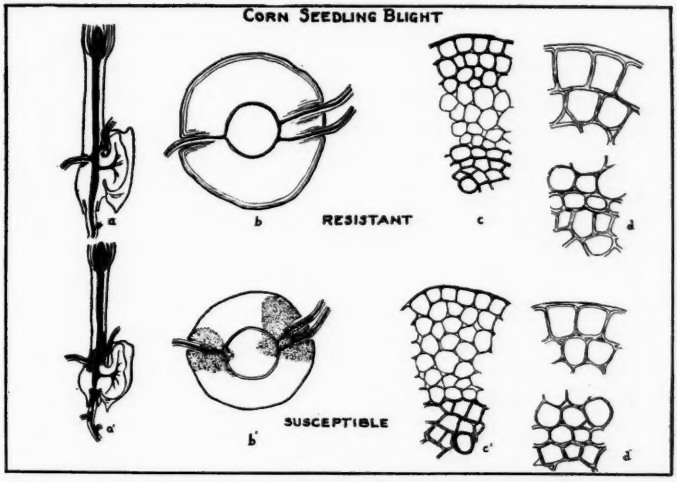

FIG. 5.—THE NATURE OF RESISTANCE TO SEEDLING BLIGHT OF CORN

SECTIONS OF RESISTANT AND SUSCEPTIBLE CORN SEEDLINGS ARE SHOWN IN A AND A′. NOTE THE PRIMARY ROOT AND ADVENTITIOUS ROOTS WHICH ORIGINATE IN THE STELE AND BREAK THROUGH THE CORTICAL SHEATH TISSUES, THE COLEORHIZA AND MESOCOTYL, TO REACH THE SURFACE. THE RESISTANT SEEDLING DEVELOPS A RESTRICTED LESION; THE SUSCEPTIBLE SEEDLING A SPREADING INVASION AROUND THESE ROOT RUPTURES. THIS IS SHOWN IN GREATER DETAIL IN B AND B′ CROSS SECTIONS OF THE MESOCOTYL AT THE POSITION OF ADVENTITIOUS ROOT FORMATION. THE RESISTANT SEEDLING HAS FORMED A CORK-LIKE BARRIER OF SUBERIN STOPPING THE INVASION WHEREAS THE PARASITE HAS SPREAD EVEN WITHIN THE CENTRAL STELE IN THE SUSCEPTIBLE SEEDLING. THE LARGER SECTIONS OF THESE MESOCOTYLS, C AND C′, SHOW THE DIFFERENCES IN CELL WALL COMPOSITION OF THE TWO LINES OF CORN. NOTE THE HEAVY WALLED OR SUBERIN REINFORCED WALLS ARE THREE ROWS THICK IN THE CORTEX OF THE RESISTANT IN CONTRAST WITH ONLY ONE IN THE SUSCEPTIBLE. ALSO THE ENDODERMIS, THE FIRST RING OF HEAVY WALLED CELLS BEYOND THE THIN WALLED CORTEX, IS CONTINUOUSLY THICK WALLED IN THE RESISTANT AND ONLY SLIGHTLY SUBERIZED IF AT ALL IN THE SUSCEPTIBLE. THE DETAILS OF THESE WALL DIFFERENCES ARE GIVEN IN D AND D′. THE RESISTANCE OF THESE WALLS TO DISSOLVING SUBSTANCES IS SHOWN IN FIGURE 6.

stages of germination, for instance, after the pericarp or fruit coat is broken, these cells prevent the disease producing organisms from entering unless the environmental conditions during ripening of the seed and also the early stages of germination have been very unfavorable. The

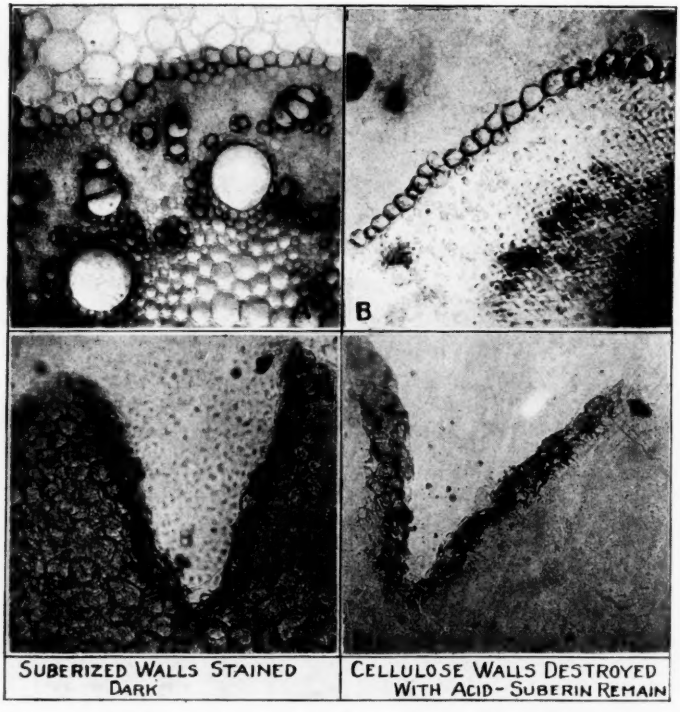

FIG. 6.—THE CELL WALL BARRIERS OF THE RESISTANT CORN SEEDLING

THE OUTER CORTICAL CELLS FORM A CORK-LIKE SUBSTANCE, SUBERIN, IN THE SURFACE LAYERS OF CELLS AND ALSO AROUND WOUNDS IN THE CORTEX. THE LOWER, LEFT HAND SECTION (C) SHOWS A NEEDLE PUNCTURE WITH THE SUBERIN-IMPREGNATED WALLS STAINED DARK. THE RIGHT HAND SECTION (D) SHOWS THIS SUBERIN BARRIER STILL INTACT AFTER THE OTHER WALLS HAVE BEEN DISSOLVED WITH SULFURIC ACID. THE STELE IS PROTECTED BY A CYLINDER OF CELLS ONE LAYER THICK SHOWN STAINED DARK JUST INSIDE THE THIN-WALLED INNER CORTICAL CELLS IN (A). THE OPPOSITE SECTION (B) SHOWS THIS SUBERIZED ENDODERMIS AFTER THE CELLULOSE WALLS HAVE BEEN DISSOLVED AWAY WITH SULFURIC ACID. THESE SUBERIN-REINFORCED WALLS ACT AS BARRIERS TO FUNGUS PENETRATION IN THE RESISTANT SEEDLINGS. THE SUSCEPTIBLE SEEDLINGS FORM THESE BARRIERS TO ONLY A VERY LIMITED EXTENT UNLESS THEY ARE GROWN AT HIGH TEMPERATURES.

roots, however, must reach the surface through the cortex if they are to function and in so doing they open up avenues of invasion into the interior of the young plants.

The details of cellular response to temperature in these tissues is different at the various temperatures.

The cell walls of the protective sheath tissues are reinforced with corky or woody substances that prevent fungus penetration. The seedlings growing under the favorable temperatures form cellulose walls impregnated with suberin in corn and principally lignin in wheat. These same materials accumulate in the cell walls around the root ruptures and as a result the fungus is corked out. See Figures 5 and 6. The seedlings growing under unfavorable conditions, at high temperatures for wheat and at low temperatures for corn, do not have these reinforced walls. In fact, the walls are not even formed of true cellulose, but are intermediate pectin-like substances. The parasite grows in between the cells of these tissues, dissolving first the middle lamella and then much of the cell wall feeding upon the pectin and xylan obtained from these walls. There is no corking off around the root ruptures, and therefore each root rupture offers an easy entrance into the growing parts of the seedling and blighting results.

Seedling metabolism of two plants different

The metabolism of the seedlings of wheat and corn is influenced differently by temperature. The cereal seedling and even later development of the plant is influenced greatly by changes in either quantitative or qualitative balance in the release of reserves from the endosperm. Temperature modifies the enzyme secretion, relative rate of action of the different hydrolytic enzymes upon the reserves, and the absorption and translocation of the simpler compounds to such an extent that the metabolic balance of the seedlings is disturbed. The wheat seedlings grown at low temperatures are high in available carbohydrates, especially glucose and sucrose and low in available nitrogenous substances. The nitrogen forms apparently are combined with the carbohydrates as soon as available to form the proteins of various forms needed

in the growing seedling. There is still an excess of carbohydrates for cell-wall formation. At the high temperatures on the other hand, the hydrolysis of starch from the wheat endosperm seemingly is quite different. There is an incomplete hydrolysis of starch resulting in the accumulation of dextrin-like substances in the endosperm, and, therefore, little available carbohydrate forms to combine with the nitrogenous substances to form protoplasm or to be built into cell walls in this rapidly growing seedling. As a result, the high-temperature seedlings are thin-walled, fragile, the walls being composed largely of pectin-like substances, and comparatively high in nitrogen, especially the readily available forms such as asparagine, amino acids, etc.

Briefly, the corn seedling appears to be just the reverse of these conditions in wheat. The low temperature seedling, due to decreased enzyme activity, is low in available carbohydrates for building tissue. This results in pectin-like cell walls and an excess of readily available nitrogen, especially asparagine. The parasite then seemingly not only has no cell wall barriers to overcome, but has an abundance of pectin-like carbohydrate and readily available nitrogen as a possible food supply in these susceptible, low temperature corn seedlings. It is little wonder then that the parasite develops aggressively and kills the seedling.

The parasite grows best on pectin-like carbohydrates and asparagine. Pectin-like substances and xylan were abundant in both the susceptible wheat and corn seedlings. When these substances were used in pure form in synthetic media, there was several times more growth of the fungus produced than when glucose or sucrose were used. See Figure 7. The fungus was unable to use cellulose as a source of carbohydrate food at any temperature. Likewise asparagine produced double the growth formed from sodium nitrate or other nitrate forms tried. The growth of the fungus on these very simple media show the same general response to these single substances as

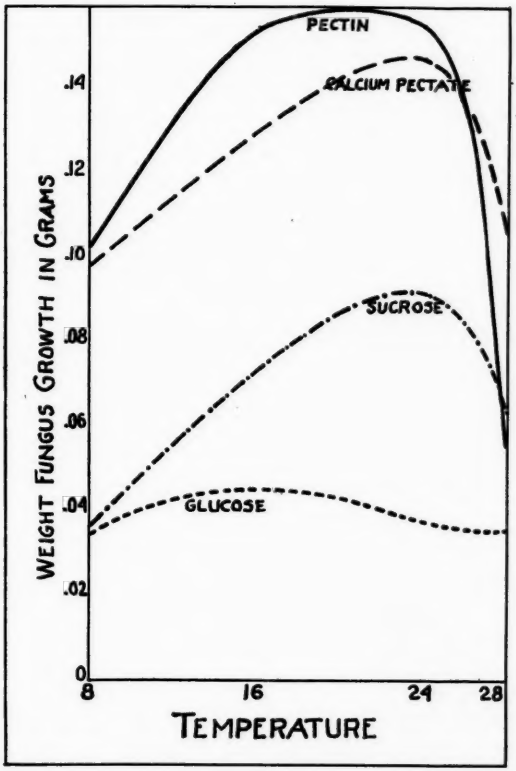

FIG. 7.—THE SEEDLING BLIGHT PARASITE USES THE SOLUBLE WALL SUBSTANCES AS FOOD

PECTIN, XYLAN AND COMBINATIONS OF THESE SUBSTANCES OCCURRING IN THE WALLS OF THE SUSCEPTIBLE SEEDLINGS FORM AN EXCELLENT FOOD FOR THE PARASITE, BETTER EVEN THAN THE COMMON SUGARS GLUCOSE AND SUCROSE.

occurred in the growth upon the plant. The complex of the available carbohydrates and nitrogen forms in the susceptible seedlings is undoubtedly an important factor in the growth of the parasite in the plant and subsequent killing of the seedling. Or, stating the converse situation, the resistant seedlings of both wheat and corn were free from disease because the reinforced cell walls prevented rapid penetration and because the nutritive substances

FIG. 8.—THE RESISTANT CORN MAKES A GOOD CROP WHERE THE SUSCEPTIBLE FAILS

SHOWING THE RESULTS OF EARLY PLANTING IN "CORN SICK" SOIL. THE RESISTANT CORN GIVES A GOOD CROP WHERE THE SUSCEPTIBLE LINE IS A COMPLETE FAILURE. ON CLEAN SOIL OR IN A WARM SOIL THE SUSCEPTIBLE STRAIN DOES AS WELL AS THE RESISTANT.

available, chiefly glucose, sucrose and cellulose with little available nitrogen, did not support fungus growth after penetration had occurred.

The influence of temperature upon the potential host plants seems to be the primary thing. The metabolic balance of the wheat and corn seedlings are changed by temperature differences. These changes in metabolism deal directly with penetrability of the host wall as determined by its composition. These metabolic modifications also determine the composition of the host cell contents and their nutritive value for fungus growth. This in turn influences the nutritive balance of the potential parasite and determines not only the rate and amount of penetration, but also the relative progress of its parasitic development after penetration.

Temperature is not the only factor of the environmental complex acting upon seedling metabolism. Soil moisture and light intensity both have been found to play a similar rôle in modifying the seedling metabolic processes and may modify the immediate effect of temperature influences or at least change the range over which they function as the dominant influence. Thus the effect of the environmental complex as a whole upon the plane of metabolism or upon the balance established within either host or parasite must be considered ultimately, rather than thinking narrowly upon one factor and its influence. The type of metabolism brought about by changed light or soil nutrients may modify considerably the temperature response of the living organism.

To return now to the fundamental question of the nature of disease resistance in these seedlings, it is a matter of especial interest to determine whether functionally the "induced resistance," as brought about by a favorable temperature as part of a favorable environment, is essentially different from that of "inherited resistance" which is expressed typically in certain resistant corn strains.

TEMPERATURE AND THE DEVELOPMENT OF DISEASE RESISTANT INBRED LINES OF CORN

Disease resistant lines of corn have been developed by inbreeding and selecting for resistance under unfavorable environments.[14] In this manner, selections have been obtained which are highly resistant to these cortical diseases, others which are intermediate and still others which are very susceptible. The resistant inbred lines develop free from disease at temperatures as low as 12° C.; the intermediate are susceptible at temperatures below 20° to 24° C.; and the susceptible are blighted at all temperatures at which the parasite grows. A study of fungus penetration and metabolism of certain of the resistant and susceptible lines has been made at the same temperatures used in the earlier experiments.

The resistant lines inhibit fungus penetration through the sheath tissues of the seedlings. The coleorhiza and cortex of the mesocotyl, the protective sheath tissues, form suberized, cellulose walls which bar the entrance of the fungus. See Figure 5. These walls are toughened by the addition of this cork-like substance into the walls at all temperatures above 12° C. Mechanical puncture of these walls requires five to ten times the pressure necessary to puncture the walls of the susceptible lines. This cork-like substance is quickly formed at root ruptures in the coleorhiza, the mesocotyl and primary seminal root, as well as around mechanical punctures of any kind, so that the parasites are prevented from entering through wounds as well as by direct penetration. Tests made upon the solubility of these suberized walls of the epidermis, the outside protective sheath and endodermis, the inside protective sheath of cells, show that they are insoluble in sulphuric or hydrochloric acids of 75 per cent. concentration. See Figure 6. The sheath cells of the susceptible seedlings, on the other hand, are digested in a few seconds in acids of the same concentration. The protective sheath tissues then act as natural barriers to prevent the entrance of any soil-borne parasite. The de-

velopment of the suberized cell walls is very pronounced in the resistant lines with an enormous reserve capacity to suberize, cork off, any injured tissue which may occur in these seedling protective or sheath-like structures.

The resistant lines maintain a balanced metabolism even at low temperatures. The suberized cellulose walls of the cortical tissues of the resistant seedlings are due largely to the high content of available carbohydrate reserve for building cell walls in much the same manner as occurred at the high temperatures in the induced resistance. The seedlings of the resistant inbred lines were high in available carbohydrate-building substances which formed cellulose walls well suberized at temperatures above 12° C. The plants, likewise, were low in uncombined nitrogenous substances over the same range of temperature; indicating essentially the same balance of reactions as occurred in the open-pollinated corn at temperatures of 24° C. Furthermore, the seedlings of the susceptible, inbred lines were high in pectin-like and xylan-like substances at temperatures below 24° C. and also high in available nitrogenous substances, again comparing favorably with the metabolic processes of the open pollinated corn at low temperatures. Inbreeding and selection under unfavorable low temperatures in the presence of the parasite in both the greenhouse and field has brought about stabilization and intensification of this capacity to function at lower temperatures during the seedling stage. See Figure 8. Likewise selection in the reverse direction, for susceptibility, has increased the tendency towards the unbalanced metabolism even at higher temperatures.

This is not a new practice in breeding, but rather an application of the methods of breeding to a more definite group of functions in plants. Resistance to seedling blight is a relative condition depending largely for its expression upon the balance of metabolism within the seedling. "Induced resistance," therefore, in this case is not essentially different from "inherited resistance." Both

depend for their expression upon the type of metabolism existing within the seedling under the environmental conditions at hand.

SUMMARY

Temperature has been shown to influence the development of the seedling blight of wheat and corn. The wheat seedlings blight in a warm soil and do not blight in a cold soil. The corn seedlings blight in a cold soil and do not blight in a warm soil. The cortical invasion and rotting was found to be due largely to an unbalanced metabolism within the seedling during the early stages of germination which resulted in the cell walls of the protective tissues being composed of intermediate, pectin-like, substances instead of cellulose well reinforced with lignin in wheat or suberin in corn. These walls were easily hydrolyzed which resulted in rapid invasion by the parasite.

The unfavorable environment was used in selecting resistant st ins. Selecting for resistance in segregating inbred l es of corn grown in cold soils infested with the parasite as resulted in resistant lines growing well at low temperatures. The further study of these lines in contrast with susceptible lines has shown that the "inheritable resistance" is similar in nature to the "induced resistance" brought about by high soil temperatures. Resistance appears to be due to an intensification and stabilization of factors controlling a balanced seedling metabolism. This results in cellulose walls well reinforced with suberin and a cell composition unsuitable for the nutrition of the parasite over a wide range of unfavorable environmental conditions.

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THE GROWTH OF COGNATE SHOOTS[1]

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THE shoots which arise from a particular branch on a tree may have a common hereditary constitution, but be so oriented in time or space that their growth processes are decidedly unequal. The greatest growth of lateral shoots is usually at the apex of an upright branch and diminishes toward the base, demonstrating what is best described as an axial gradient. This inequality in growth leads to differences in their ultimate size and to the development of a more or less characteristic shape. The immediate object of this study was to analyze and compare the growth processes of cognate shoots of the lemon (*Citrus limonia* Osbeck) which were strictly vegetative in their activity during the period of these observations. It will be important to see how far the relative size of organisms is dependent upon the presence of what Sachs termed shoot-forming substances, and how far upon the rate of metabolism. The possible action of a growth-inhibiting substance, as suggested by Loeb, is also worthy of further study. The problem of differentiation is not so much a question of the presence of organs as of their position and relative size. Horticulturists have long distinguished between vegetative and fruit-bearing wood, but more light upon the problem of their development is needed.

The data on which the study is based were secured from five-year-old lemon trees at the Citrus Experiment Station, Riverside, California, in 1922. Dr. F. F. Halma kindly assisted in obtaining the data. A few weeks before the shoots began to grow the trees had been pruned by heading back all the upright vigorous shoots of the previous season's growth. In this particular lot of trees the basal portions remaining after heading were 20 to 40

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cm. long and had from 12 to 20 dormant lateral buds. These stubs represented one fifth to one third of the original length of the shoots. Three dormant buds on each stub were marked with suitable labels late in April, and the growth from each was measured as soon as it reached the length of one centimeter. For the purpose of this study the increase in length will be regarded as the measure of growth. Measurements were taken at weekly intervals from May 8 to August 28, then at biweekly intervals until growth ceased on October 23, covering a period of twenty-five weeks. The three buds selected were representative, so far as possible, of the upper, middle and lower part of the shoot-producing area. The

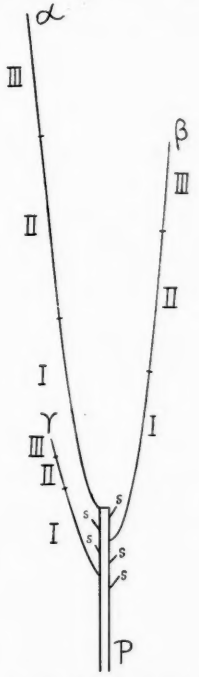

FIG. 1. Diagram of a representative parent branch (P) with three of the cognate shoots (α, β, and γ). S, S, other cognate shoots. Roman numerals show portions of shoot produced during the first, second, and third cycles, respectively.

shoot near the apical or distal end of the mother shoot was α, the β shoots were midway, and the γ shoots nearest the basal or proximal end. There were ninety-two shoots in the α class, ninety-eight in the β, and ninety-two in the γ. Their distribution will be evident from the statement that the mean ordinal positions of α, β, and γ shoots were 1.65, 4.28 and 7.51, respectively. The mean number of shoots per mother shoot was 7.95, whence it is readily seen that the distribution represented the apical, median and basal positions. The mean length of the shoots at the end of the growing season was 92.32 cm, 69.93 cm and 24.96 cm, respectively, for the three classes. The diagram (Fig. 1) shows the relative length of the average shoot in each class at the end of the growing season and indicates their distribution on the parent branch.

I. GROWTH OF THE POPULATION OF SHOOTS

Each class of shoots had a three-cycle growth during the season of 25 weeks. The cycles were practically equal in length (8 weeks) and were synchronous for the three classes. These cycles necessarily overlapped to a slight degree, but each was entirely distinct. The curve of growth for the season is a summation of the curves of the three cycles.

The values of the mean length of shoots at the weekly intervals lie very close to the values calculated from the equation frequently used in studying the growth processes of various organisms (Robertson, 1923).

$$\log \frac{x}{A-x} = K\,(t-t_1)$$

where x = length of the shoots at time t; A = final length (limiting value of x); t_1 = time at which $x = A/2$, the point of inflection of the curve; and $K \equiv$ a constant. The length as computed from this equation is an exponential function of the time.[2]

The equations for the growth curves of the cycles are as follows:

[2] It is a pleasure to acknowledge the help of my wife, Hannah Dewey Reed, in making the computations which follow.

α shoots

first cycle, $\log \frac{x}{36-x} = .411$ (t–3.85)

second cycle, $\log \frac{x-34}{69-x} = .336$ (t–11.75)

third cycle, $\log \frac{x-67}{93-x} = .311$ (t–19.0)

β shoots

first cycle, $\log \frac{x}{32-x} = .433$ (t–3.5)

second cycle, $\log \frac{x-30}{56-x} = .325$ (t–11.16)

third cycle, $\log \frac{x-53}{70.5-x} = .292$ (t–18.4)

γ shoots

first cycle, $\log \frac{x}{17.3-x} = .438$ (t–3.14)

second cycle, $\log \frac{x-16.5}{22.76-x} = .305$ (t–11.0)

third cycle, $\log \frac{x-22.5}{25.5-x} = .253$ (t–19.4)

The general correspondence between the cyclic growth (Figures 2, 3 and 4) of the three classes of shoots indicates that their growth processes were of the same general nature although the constants differed somewhat in each class of shoots. The final length of each class of shoots (represented in the equation by A) was dependent upon the amount of material suitable for growth which flowed into the shoots. In the first cycles the values of A were larger than in succeeding cycles. Its decline in value in successive cycles was most marked in the case of the γ shoots and least in the α. There are many factors which influence growth, but there seems good reason to assume that the shoots on the apical part of the branch attain and hold their dominance by drawing upon the supplies of growth-producing materials in the parent branch as well as by inhibiting the growth catalysing substances in subapical growth centers.

The values of $\frac{A \times 100}{\Sigma A}$ are percentages of the final length produced in any given cycle. It will be seen that the γ shoots made 65 per cent. of their total growth in the first cycle (Table I) and that the α shoots, although they attained a greater length, made 37 per cent. of their

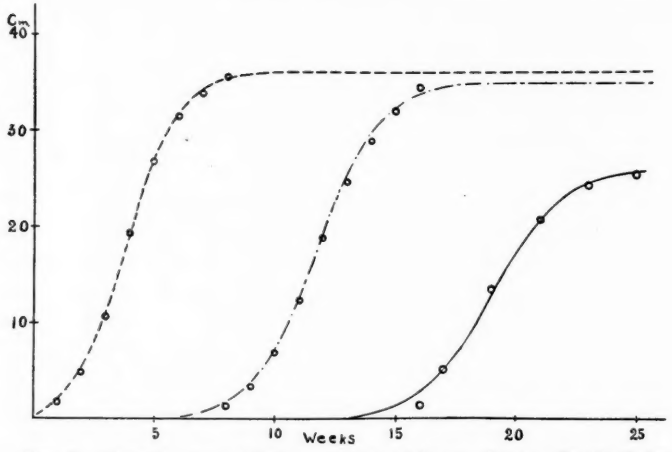

FIG. 2. Growth curves of the α group of lemon shoots. Small circles represent observed values. Curves represent calculated values: – – –, first cycle; — — —, second cycle; ———, third cycle.

growth in the first cycle and 27 per cent. in the last cycle. These values may be interpreted to mean that the substrates suitable for growth were more abundant early in the season and that the subapical shoots were more restricted in their growth by the failing supply as the successive cycles came on.

The values of t_1 for the first cycle of the successive groups were 3.85, 3.50 and 3.14 weeks, respectively. One can not strongly emphasize such differences as these, but they suggest that the more vigorous shoots required somewhat more time to reach their half-length in the first cycle than their neighbors. In other cycles there were no pronounced differences.

The values of K, the constant of the equation, are of much importance since they represent the magnitude of a specific function of the growth process. The slope of the growth curve depends on the value of K (Figs. 2, 3 and 4). A steep curve is associated with larger values of K.

The values of K for the several groups were less in successive cycles of growth. When values of K were plotted

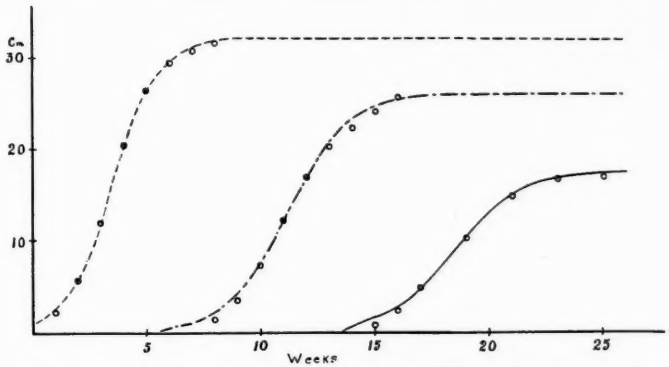

FIG. 3. Growth curves of the β group of lemon shoots. Small circles represent observed values. Curves represent calculated values: – – –, first cycle; — – —, second cycle; ———, third cycle.

as ordinates and values of A as abscissae the relations were quite evident, although there were only three points for each graph (Fig. 5). The graphs are not spaced uniformly with respect to values of A because, as previously shown, there was more similarity between α and β groups than between β and γ groups. The slope of the graphs shows a close parallelism, however, indicating that in each cycle the velocity constants of the growth processes of different groups of shoots were determined in a very similar way.

The experimentally determined values of K in this as in most other cases, were larger at the outset of each cycle and declined, at first rapidly, then more slowly as the cycle progressed (Reed, 1920a, 1928). The values of $\frac{x}{A}$ (the relative size at any given time) appear to be an exponential function of K. We must not overlook the fact that $K = Ak$, and that we were comparing A with one of its functions. The true velocity constant is k. Reference to the graphs in Figure 7, however, will show that the statement is true for k. This relation of the velocity constant to relative size must be an expression of the activity of the growth-promoting substance in the shoot.

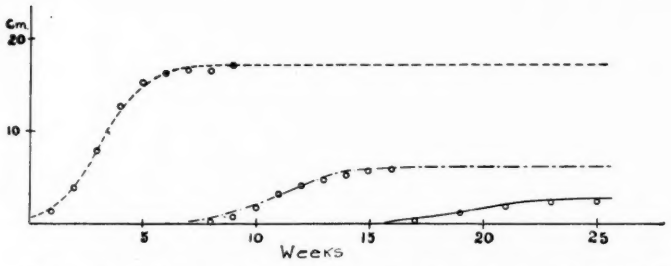

FIG. 4. Growth curves of the γ group of lemon shoots. Small circles represent observed values. Curves represent calculated values: – – –, first cycle; — – —, second cycle; ———, third cycle.

Robertson (1926) demonstrated a striking correlation between the decline in the velocity and the nucleo-cytoplasmic ratio in mice and concluded "that the velocity-constant of autocatalysis in the growth of mice is proportional or nearly proportional to the nucleo-cytoplasmic ratio." The possibility also exists that there were two velocity constants K_1 and K_2 unequal in value. Crozier (1926–27) has proposed a formula which represents a growth process of this kind.

The significance of k, the true velocity constant, is seen from the differential form of the equation for growth or autocatalysis

$$\frac{dx}{dt} = k\,x\,(A - x)$$

The equations for the growth of the α and β classes show a comparatively narrow range for the values of k with respect to A, though there was a slight increase in these values in the later cycles.

The mean value of K for the first cycle of the γ shoots was .438, which is probably significantly higher than the corresponding value of K for the α shoots, if not for the β shoots. Although the difference is not great, it agrees with the difference in the values of t_1 already mentioned, and confirms the idea that the γ shoots grew relatively faster in the first cycle than the shoots of either of the other classes.

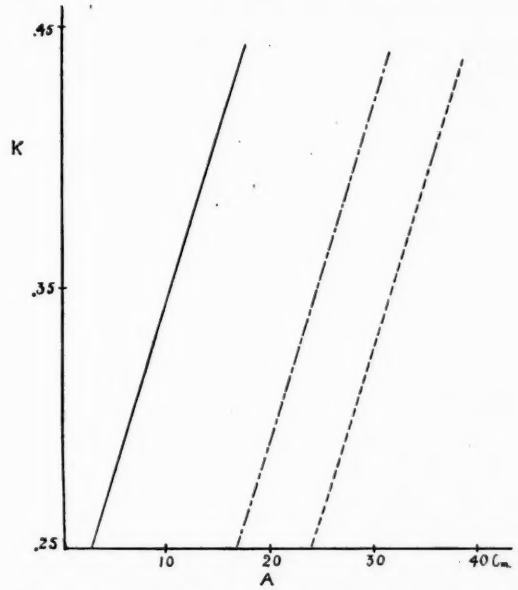

FIG. 5. Graphs representing the relations between the mean value of K and A for each group of lemon shoots. – – –, α group; — – —, β group; ——— γ group.

Another illustration of the relation between the mean value of K and of $\frac{A \times 100}{\Sigma A}$ is shown in Figure 6. The log values of K plotted against the fraction of the final growth attained in that cycle give very good graphs whose slopes decrease from group α to group γ. The slopes resemble the mean slope of the curves of growth in figures 2, 3 and 4. From what has been said, it is apparent that there is a homogeneity in the physiological functions of these classes of vegetative shoots which is manifested by the constants of their growth curves as well as by the similarity of the products of the growth reactions (Reed, 1921a).

The length of the shoots in any group appears to depend upon the amount of nutrient substance they can acquire during the growth period. The amount, in turn, may depend upon the rate at which reserve materials may

TABLE I

CONSTANTS OF THE GROWTH EQUATIONS OF LEMON SHOOTS

Class	α			β			γ		
Cycle	I	II	III	I	II	III	I	II	III
Total population:									
Values of A (cm)	36.0	35.0	26.0	32.0	26.0	17.5	17.3	6.26	3.0
Values of t_1 (weeks)	3.85	11.75	19.0	3.50	11.16	18.40	3.14	11.00	19.40
Mean value of K	.411	.336	.311	.433	.325	.292	.438	.305	.253
Root-mean-square deviation of calculated from observed values of x (cm)	.43	.59	.57	.31	.55	.34	.34	.26	.29
Values of $\frac{A \times 100}{\Sigma A}$	37	36	27	42	35	23	65	24	11
"One-cycle" shoots:									
Values of A (cm)	29.0			23.0			15.0		
Values of t_1 (weeks)	3.70			3.20			3.00		
Mean value of K	.473			.530			.471		
Root-mean-square deviation of calculated from observed values of x (cm)	.48			.40			.74		
Population exclusive of "one-cycle" shoots:									
Values of A (cm)	37.0	37.0	31.5	36.0	34.0	22.0	25.6	25.6	11.6
Values of t_1 (weeks)	3.85	11.66	19.00	3.60	11.30	18.90	3.40	11.00	19.10
Mean value of K	.471	.418	.361	.416	.348	.300	.446	.349	.261
Root-mean-square deviation of calculated from observed values of x (cm)	.55	.74	.67	.49	.90	.70	.32	1.10	.66
Values of $\frac{A \times 100}{\Sigma A}$	35	35	30	39	37	24	41	41	18
Shoots on which laterals were produced:									
Values of A (cm)	39.0	39.0	32.5	37.5	31.0	22.9			
Values of t_1 (weeks)	3.90	12.22	19.00	3.68	11.72	19.05			
Mean value of K	.411	.409	.369	.459	.405	.303			
Root-mean-square deviation of calculated from observed values of x (cm)	.58	.61	1.09	.25	.55	.93			
Values of $\frac{A \times 100}{\Sigma A}$	35	35	30	41	34	25			

be transformed into appropriate compounds. Data presented in an earlier paper (Reed, 1920b) showed that the constants of the equations for curves of slow and rapid growth were the same, but that the values of A in the two equations differed consonantly with the amount of growth. The data from the lemon trees now under discussion afford very substantial confirmation of the conclusions previously drawn because they afford an example of the unequal growth of shoots arising from neighboring buds on the same mother shoot.

II. One-cycle Shoots

Each of the three classes under discussion included a number of shoots which grew only during the first cycle. The α class contained 17 shoots of this kind, the β class 28, and the γ class 71. These shoots produced no laterals or other form of later shoot growth during that season and will be here designated as "one-cycle" shoots. Their behavior presents a problem of some interest. The factors which inhibited the growth of a portion of the shoots after they had made seven or eight weeks of active growth would profoundly alter the character of the tree if they similarly affected all the shoots. At the same time we realize that this "one-cycle" type of shoot is always found on the lemon tree. Its subsequent history indicates that many of them develop fruiting, rather than vegetative, activities. Their number was greatest in the γ class and smallest in the α class which was predominantly vegetative. Their final mean length in the three classes showed a progressive decline from 29 to 15 cm (Table I) somewhat similar to that of the general population, though not parallel. The mean values of K (the constant of the equations) are not greatly different from those of the first cycle in each of the respective groups of the general population. The same may be said of the values of t_1. So far as the values of these constants are concerned, one can see no striking difference between the one-cycle shoots and the first cycle of growth of their

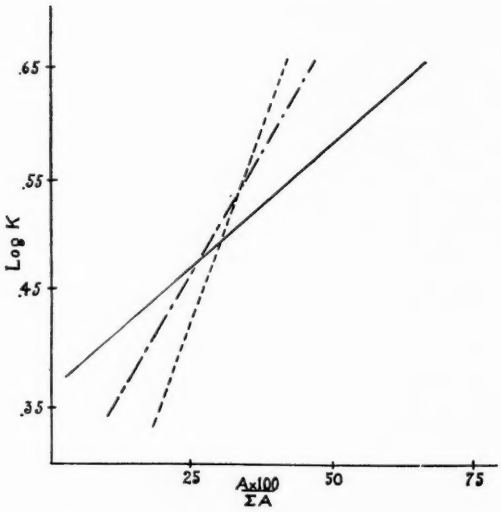

FIG. 6. Graphs representing K as an exponential function of $\frac{A \times 100}{\Sigma A}$
– – –, α group; — – — –, β group; ———, γ group.

three-cycle neighbors. The differences must be sought elsewhere.

There are many agents which affect growth, yet most of them act either by changing the amount of material which enters into the transformation, or by affecting the mechanism of the transformation process. There is no evidence that the first of these possibilities is operative in this case. The mean final length of the one-cycle shoots is not so far below that of their neighbors as to indicate that the quantity of appropriate materials was responsible for the cessation of growth. Restricted nutrition, so far as known, does not terminate growth, unless it terminates life. Tomato plants grown by Murneek (1926) with small amounts of nitrate grew as long as those given larger amounts although the mean final length was less in the former lot of plants. Young white rats which were maintained on very restricted diet made a small amount of growth if they lived and grew to full size when given an adequate ration (Reed, 1921c). Apri-

cot shoots which grew slowly and reached a mean length of 100 cm had as long a growing period as those on neighboring trees which reached a mean length of 218 cm (Reed, 1920b).

The alternative question relates to the mechanism of the process, *i.e.*, the autocatalyst. Results obtained at this laboratory have a bearing upon the point. A cutting from a tree like the Chinese lemon when suspended in a horizontal position produces shoots only from buds on the upper surface (Reed and Halma, 1919). If the cutting be rotated through 180° after shoots have begun to grow, the downward pointing shoots soon cease growth. Later the buds on the opposite (upper) side of the cutting may grow into new shoots. A horizontal shoot of a lemon tree shows similar distribution of growth on the dorsal side (Halma, 1926). If the branch be reflexed after the shoots begin to grow so it is again horizontal, but with the shoots on the ventral side, they finish their growth cycle, but grow no further, but a second set of shoots will grow from the buds which have been brought uppermost by reflexing the branch. Obviously there was a factor in the ventral side of the horizontal branch or cutting which inhibited the further growth of shoots which had already started. When buds on the dorsal side of a branch were killed or their growth continually removed the buds on the ventral side still suffered complete inhibition.

The stoppage of growth when the horizontal member was turned is evidence of the action of some factor which terminated the growth process. In a somewhat similar way the growth of these one-cycle shoots was stopped by a factor antagonistic to their growth, and it is more logical to believe that something stopped the action of the growth catalyst than that the supply of nutrient substances suddenly failed.

Had these one-cycle shoots been pruned back after August first, new growth would have arisen from some of them, indicating that the materials necessary for growth were present.

If the difference between one-cycle and three-cycle shoots is mainly one of their growth catalysts it may be profitable to consider the values of k, which I have assumed to express the activity of the catalyst. Inspection of Figure 7 will show the observed values of k for the first cycle of the three-cycle shoots compared with those of the "one-cycle" shoots. Each is plotted against the values of x/A for the cycle and therefore represent equivalent stages of development. Data for shoots in the α class were not included because of the small number of "one-cycle" shoots in that class. The values of k in the first cycle of the three-cycle shoots are very uniform until the cycle is more than 75 per cent. complete, then they drop, but not far. The values of k in the equations of the "one-cycle" shoots present quite a contrast. They increased in value until the shoots had made about 75 per cent. of their growth, and then suddenly fell to a level much lower than their original values.

These fluctuations in the value of k may be interpreted as fluctuations in the activity of the catalyst. Accordingly we should conclude that some antagonistic agent was the cause of the rapid decline of the activity of the catalyst and that its intensity was great enough to prevent subsequent recovery in these one-cycle shoots.

There is evidence that the size of an organ is some function of its position on the longitudinal or radial axis which bears it (Penrose, 1925; Reed, 1921b, 1924; Pearl, 1907). It is suggestive to note that there is a fairly constant ratio between the one-cycle shoots in each class and the per cent. of one-cycle shoots in the class. It turns out that the ratios are 11.2, 6.7 and 10.3. It is not believed that this constancy is due merely to the chance operation of factors which are mainly outside the plant. On the contrary there is everything to indicate that factors of a hereditary character wield a dominant influence upon the termination of growth at the end of the first cycle.

III. Shoots on which Laterals were Produced

Laterals were produced upon many of the shoots in groups α and β in the latter part of June, and many of them made considerable development during the remainder of the season. A study of the mean length of these shoots indicates that they were among the superior members of the population discussed.

Some of the trees were slightly affected with the rather common malady known as foliocellosis, or "mottle-leaf," but this did not seem to affect the production of laterals. The α and β groups contained a total of 193 shoots, 98 of which produced no laterals and 95 produced laterals be-

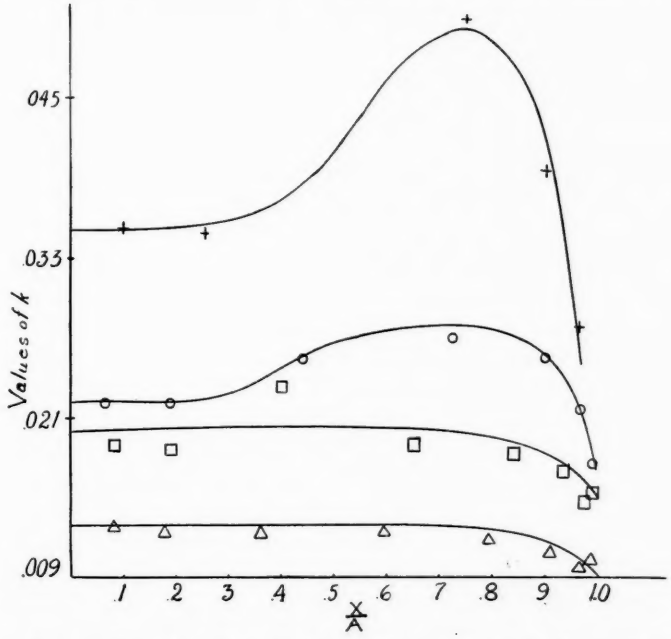

Fig. 7. The values of k in the equations of shoots of the β and γ groups, showing the differences between the "one-cycle" shoots and the three-cycle shoots. Curves drawn by inspection. △ △, β group, three-cycle shoots; ⊙ ⊙, β group, "one-cycle" shoots; □ □, γ group, three-cycle shoots; + +, γ group, "one-cycle" shoots.

tween July 17 to 30. The mottled shoots were 33 per cent. of the first class and 30 per cent. of the second class. The small difference indicates that mottling was not a factor in the production of laterals on these shoots. So few of the γ shoots produced laterals that they are not included in this part of the discussion.

It will readily be noted that the length of shoot produced in each cycle was in excess of the average of the general population (Table 1) although the duration of the respective cycles correspond rather closely. The length of the two groups of shoots on June 12 (just before laterals appeared) was 32.27 cm for the α shoots of the general population and 35.28 cm for the same group in the population which later produced laterals. In the case of β shoots the lengths were 29.81 cm and 34.94 cm, respectively. In each case it is apparent that the shoots which produced laterals were larger from an early stage. A similar relation exists in the apricot (Reed, 1924). The proportion of growth produced in each cycle was practically the same as that of the shoots after excluding the "one-cycle" shoots. In fact many of the same shoots were included in these two groups.

There is more evidence from the constants of the growth equations that these lateral-bearing shoots had larger supplies of growth-producing materials than that there was any consistent difference in the amounts of growth-promoting catalysts.

IV. Discussion

The foregoing data on the growth of cognate shoots show that they reach their final size by processes of growth which are essentially similar in nature. The important problem is the axial distribution of growth on the parent branch. Do the longer shoots owe their superior length to a more active growth-promoting substance or to a larger supply of the materials which enter into the formation of these particular organs? The evidence favors the idea that the catalyst distributed among the buds on the shoot is homogeneous. Halma (1926) has shown that

so simple an operation as making a notch in the cortex of a lemon shoot will often result in the growth of an otherwise dormant bud. Denny (1926) showed that minute amounts of ethylene, thiourea and other substances may initiate growth of dormant buds of certain plants and tubers. The treatment shortens the rest period by releasing a catalyst or starting a zymatic action. If these treatments release the catalyst or convert reserve materials into growth-producing materials they would abbreviate the rest period, by hastening a process which would eventually occur without their action. Under natural conditions the upper buds are the first to grow out, indicating that the transformation of food reserves begins at the apex of the shoot. Having begun to grow, they draw soluble substances from subapical regions, depleting by that amount the substrate. Halma (1926) has shown that the transformation of growth-producing substances in lemon branches can proceed when the buds are wrapped with tape which mechanically prevents the buds from growing into shoots. Gardner (1925) has found that starch begins to disappear from the apical part of a shoot as the spring growth begins and progressively disappears downward. Jones (1925) found that a difference of no more than 2° C. was sufficient to cause dominant growth in a region which was otherwise suppressed.

The growth constants of the β and γ shoots plainly imply that there was no impairment of their catalysts, but that their supply of growth-producing substances was reduced below that available for the α shoots.

V. Summary

1. The existence of an axial gradient of growth in lemon trees was demonstrated by the regular arrangement of cognate shoots on the parent branch. The longer shoots appear to owe their superiority to the presence of a larger amount of the appropriate substrates in the apical portion of the branch, or at least, to a more rapid transformation of reserve materials into growth-produc-

ing materials in that region. The superiority of the apical shoots may ultimately rest on the activity of some catalyst which effects the transformation of reserve materials.

2. Each group of shoots had three well-defined cycles of growth during the annual growth period. The increments in successive cycles were more nearly equal in the case of the larger shoots than in the smaller. The mean velocity constants of the various cycles were logarithmic functions of $\frac{A \times 100}{\Sigma A}$ (the per cent. of total growth produced in a given cycle) and arithmetical functions of the actual growth of a cycle.

3. In each group of shoots there were some which terminated their growth for the season at the end of the first cycle. The growth of these shoots suggested that the activity of their growth-promoting substance was completely stopped by some inhibitory substance which became active toward the end of the first growth cycle.

4. The larger shoots had a tendency to produce laterals and to maintain their initial superiority throughout the season.

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THE EFFECTS OF X-RAYS ON PRODUCTIVITY AND THE SEX RATIO IN DROSOPHILA MELANOGASTER

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INTRODUCTION

USING Muller's X-ray dosage by which he was able to induce numerous mutations in Drosophila, a study was made of the daily egg production in irradiated flies, the mortality rate in eggs, larvae and pupae, the differences appearing in the sex ratio, and visible mutations.

Two dosages known in Muller's laboratory as the T-2 and the T-4 were used in these experiments. The T-4 is twice the strength of the T-2 and is, apparently, about the optimum dosage for the production of mutations, as dosages much higher than this render too great a percentage of sterile flies to be practicable.

MATERIAL AND METHODS

The males of a stock having forked bristles and Bar eyes (fB) were given the two treatments mentioned above in lots of thirty each. An equal number of untreated males from the same stock served as controls. Each male of the two treatment groups and the controls was put into a four by one inch test tube with an *untreated* virgin female which was wild type in all respects except for tinged eye color (w^t). Food was given each pair by inserting into the test tube a paper spoon (confectioner's ice cream spoon) into the bowl of which had been poured food made according to the well-known banana-karo-agar-yeast formula used by most Drosophila workers. These spoons were allowed to remain

in the tubes for twenty-four hours, after which they were removed and a fresh spoon of food put in. Immediately upon removal of each spoon from the test tube the eggs laid during the past twenty-four hours were counted and recorded.

There were, as will be understood from the above description, thirty tubes each of the controls, the light treatment group (T-2) and the heavy treatment group (T-4). Egg counts were made daily upon these ninety pairs of flies for a period of six days.

Upon the completion of each egg count the spoon was put into a half-pint milk bottle containing the usual food to await the hatching of the eggs. Seventy-two hours after the first egg count was made the spoon was again examined and a record was made of all unhatched eggs. The difference between the first egg count and the second seventy-two hours later was considered to be the number of viable eggs laid during that twenty-four hour period. Of the unhatched eggs after seventy-two hours, an occasional one may have hatched later, but the data show that this was very rare and occurred with about equal frequency in all three groups.

The paper spoons were dipped in melted paraffin before the food was poured on. After the second egg count the mass of banana-agar was lifted from the spoon and laid on top of the food in the same bottle in which it had spent the preceding seventy-two hours. The larvae soon crawled out on this new food and completed their life history in the bottles.

Since the possible disturbance of the sex ratio was one of the objects of the experiment a careful count of the flies hatching in each bottle was made. Inspection of the bottles and fly counts was made until nearly time for the second generation to emerge. A great point was made of getting all the flies that hatched and it is highly probable that this was successfully accomplished.

The second egg count gave the egg mortality in the three groups, and by subtracting the number of flies that

hatched from the number of eggs that hatched into larvae, the combined mortality for the larval and pupal stages was obtained. It would be difficult to separate the larvae and pupae death-rates under the conditions of this experiment. It is certain, however, that the death-rate in the pupal stage is very small. When the pupa stage is reached by any individual it is pretty apt to complete its life history.

The writer is on leave of absence from Washington University for the first semester of 1927–28, and spent the second half of the semester in the zoological laboratory of the University of Texas. The problem was suggested by Professor H. J. Muller, and he also made many helpful suggestions during the progress of the work. To him and also to Professor J. C. Patterson, who put the facilities of the laboratory at my disposal, my hearty appreciation is extended.

EXPERIMENTAL RESULTS

Egg Mortality: Table 1 and Fig. 1 give the results of the egg counts for the six days. It will be seen that there is a heavy death-rate of eggs in the T-4 or heavy treatment group. On the third day 71.3 per cent. of the eggs

TABLE 1

A TABLE SHOWING NUMBER OF EGGS LAID AND EGG MORTALITY FOR DAILY COUNTS OF SIX DAYS. T–4, HEAVY TREATMENT; T–2, LIGHT TREATMENT; AND CONTROLS

	T–4			T–2			Controls		
Days	Eggs	Died	Per cent.	Eggs	Died	Per cent.	Eggs	Died	Per cent.
1	471	344	73.0	493	240	48.6	904	185	20.4
2	900	690	76.6	728	358	49.1	1406	296	21.0
3	923	659	71.3	1004	502	50.0	1336	184	13.7
4	1135	844	74.3	1147	708	61.7	1499	289	12.6
5	1162	923	79.4	1019	639	62.7	1150	254	22.0
6	1277	1081	84.6	1305	874	66.9	1322	354	26.7
Total	5868	4541	77.3	5696	3321	58.3	7617	1562	20.5

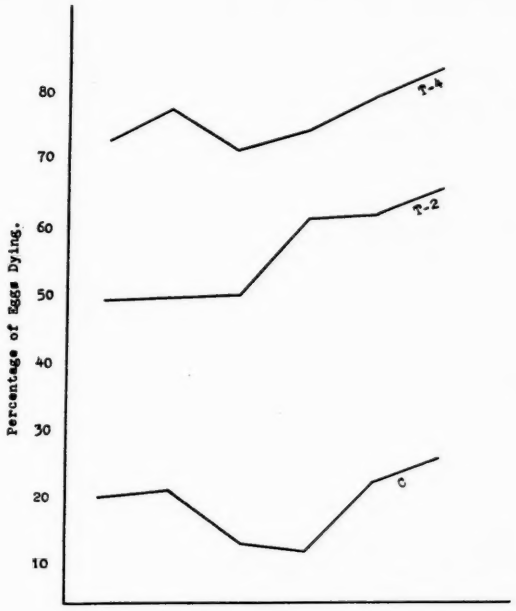

Figure I.

T-4 = Light Treatment.
T-2 = Heavy Treatment.
C = Controls.

laid failed to hatch into larvae, while on the sixth day 84.6 per cent. did not hatch. The other four days have a mortality rate falling between these two extremes. When the death-rate of the eggs for the six days is totaled, 77.3 per cent. of all eggs laid by females mated to heavily irradiated males are found to die in the egg stage.

The length of time males are exposed to the action of the rays seems correlated with the percentage of eggs dying. In the T-2 group, the mortality falls to 58.3 per cent. for the total of the six days, with a range of mortality from 48.6 per cent. on the first day to 66.9 per cent. on the last day. The difference in the two totals, 77.3 for the T-4 and 58.3 for the T-2, is clearly significant statistically.

The egg mortality in the controls is 20.5 per cent. As a criterion for comparison with the two X-rayed groups it shows a valid difference between treated and controls, whether the treatment be light or heavy. But 20.5 per cent. mortality is high for untreated flies. From two to five per cent. would be nearer our expectations. Just what factors were operating to cause a 20 per cent. egg mortality in the control flies are unknown. The stocks used contained three mutant genes, tinged eye color, forked bristles and Bar eye. Mutant races are frequently less viable than the wild type, and this may be the normal egg death-rate in this stock. Then, too, there was a certain amount of disturbance in handling the spoons, counting the eggs and transferring from tubes to milk bottles, which is absent in ordinary fly breeding.

Accepting 5 per cent. as being near the normal rate of egg deaths, correction for these three groups could easily be made. But such computations would not disturb the relative mortality rates of the three groups, hence are hardly worth the trouble.

The possible cause or causes for the high mortality in the treated as compared with the controls will be discussed later in the paper.

Larvae and Pupae Mortality: The method by which the number of individuals dying in the larval and pupal stages was arrived at is described above.

TABLE 2

A TABLE SHOWING NUMBER OF LARVAE AND PUPAE DYING IN X-RAYED AND CONTROL MATERIAL

Days	Larvae	Died	Per Cent.	Larvae	Died	Per Cent.	Larvae	Died	Per Cent.
1	127	81	63.7	253	78	30.8	719	157	21.8
2	210	110	52.3	370	136	36.7	1110	215	19.3
3	264	145	54.9	502	232	46.2	1152	220	19.0
4	291	139	47.4	439	106	24.1	1210	202	16.6
5	239	128	53.5	380	102	26.8	896	127	14.2
6	196	71	36.2	431	128	29.6	968	124	12.8
Total	1337	674	50.4	2375	782	32.1	6055	1045	17.2

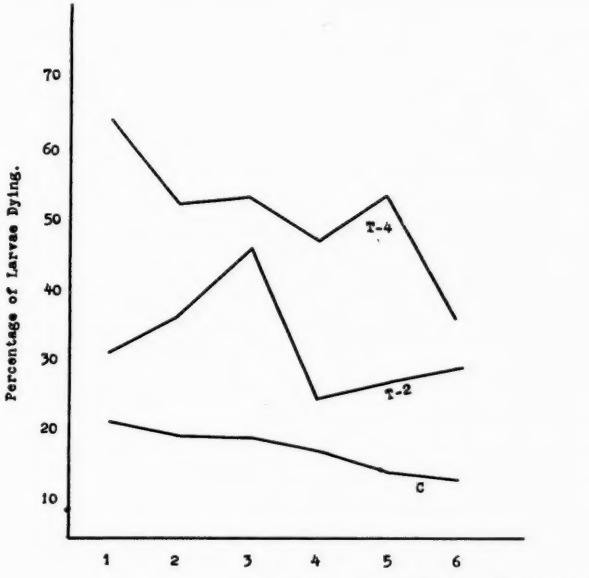

Figure II.

Table 2 and Fig. 2 give the results of this part of the study. 50.4 per cent. of the eggs that hatch into larvae in the T-4 group die in that stage or in the pupa. Since 77.3 per cent. of the eggs laid never hatch into larvae, it follows that of all eggs laid in the heavily treated group only 11.3 per cent. complete their life history.

In the T-2 group 32.1 per cent. die as larvae or pupae, which taken together with an egg mortality of 58.3 per cent. gives an adult population of about 14 per cent. of the total number of eggs laid.

The controls have an egg death-rate of 20.5 per cent. Of the 79.5 of viable eggs 17.2 per cent. die in the later stages, hence over 67 per cent. of the control eggs reach the adult stage, as compared with 11 per cent. in the T-4 and 14 per cent. in the T-2 groups.

DISCUSSION OF DEATH-RATES

The facts brought out in this experiment with regard to mortality rates in eggs, larvae and pupae are definite and clear-cut. Moderate doses of X-radiation significantly increase the death-rate of eggs, larvae and pupae; while a heavy dosage is still more disastrous in its effects. But the interpretation of these facts is not so simple. What factors are operating to produce this high mortality? What is the method whereby it is determined that some zygotes shall die in the egg stage, others in the larval stage and still others in the pupal stage? This is a problem for the future. At present it may be profitable to speculate a little as to just what happens, and this may indicate the line of attack.

Since X-rays are definitely known, from Muller's (in press) experiments, to cause lethal mutations it might be inferred that the treatment of the sperm had produced dominant lethal mutations which killed some zygotes in the egg stage and others later.

An alternative hypothesis may be found in the well-known fact that the sperm in addition to carrying into the egg the paternal set of genes also functions in stimulating the egg to develop. Although there is no direct evidence for it at the present time it may be that the act of copulation and the presence of sperm in the female act as a stimulus to egg laying. For virgin females lay few or no eggs. Now if the X-rays had coagulated or inactivated the chromosomes in the head of the sperm its function as a stimulating agent might remain, although its chromosomes would be incapable of entering into the fertilization process. In such an event a cytological study might reveal in the history of the sperm nucleus within the egg why only 11.3 per cent. (T-4) of the eggs reach the adult stage. Eggs that are destined to die may never have formed a fertilization nucleus and would, therefore, be haploids. (The Hertwigs have some evidence on this point from the Amphibia.) Or if the eggs are actually diploids the chromosomes of the sperm may

have undergone physical or chemical changes such that an incompatibility is set up and orderly development becomes impossible.

Drosophila cytology is admittedly difficult, but the eggs have been sectioned (Huettner, 1923, 1924), and this seems the most promising means of solving this problem at the present time.

The Sex Ratio

With such a tremendous mortality of eggs, larvae and pupae in the X-rayed groups the presumption seemed valid that there might be a differential mortality between males and females. Therefore a count of the entire hatch was made, special precautions being taken to record all the flies emerging.

Tables 3 and 4 give the complete data and summary of the sex ratio results. Since the flies in the treated groups were fewer in number than the controls, the treated pairs were put into new food bottles and allowed to continue breeding for an additional six days. The results of this second six days' breeding are given in Table 4 as "addi-

TABLE 3

A Table Showing the Sex Ratio Data of F_1's from an X-rayed Male Parent and Untreated Mother

	T–4			T–2			Controls		
Days	Males	Females	Sex-ratio	Males	Females	Sex-ratio	Males	Females	Sex-ratio
1	19	27		75	73		288	276	
2	51	59		108	109		449	452	
3	65	49		138	137		430	481	
4	75	73		162	171		491	516	
5	51	56		150	152		383	397	
6	69	56		167	133		418	426	
Totals	330	320		800	775		2459	2548	
Additional Data	224	203		417	435		.....	.....	
Totals	554	523	51.4	1217	1210	50.2	2459	2548	49.1

TABLE 4

A TABLE SHOWING DIFFERENCES AND PROBABLE ERRORS OF THE SEX-RATIO CONSTANTS OF TABLE 3

	Diff.	$\frac{\text{Diff.}}{\text{P.E.}}$
T–4 minus controls	$51.4 \pm 1.03 - 49.1 \pm 0.47 = 2.3 \pm 1.13$	2.03
T–2 minus controls	$50.2 \pm 0.68 - 49.1 \pm 0.47 = 1.1 \pm 0.83$	1.3
T–4 and T–2 minus controls	$50.5 \pm 0.57 - 49.1 \pm 0.47 = 1.4 \pm 0.74$	2.02

tional data," and all computations and comparisons with the controls include these data.

The actual difference between any two of the groups is small (Table 4). And when the differences are divided by their probable errors none of them shows a statistically valid difference. The difference between the sex ratio of the T-4 group and the controls is only 2.03 times its probable error; that between the T-2 and the controls is 1.3; while combining the T-4 and T-2 groups and comparing with the controls gives only 2.02.

Muller (in press) has data on the sex ratio in offspring of irradiated parents which give statistical differences of greater magnitude than these, but still just under the accepted three times the probable error. However, his data are in the same direction as these reported here and with such a great mortality of eggs and larvae coupled with complete consistency of all data available leads us to believe that the suppression of the females in the treated groups may have a biological significance in spite of the fact that it can not be demonstrated biometrically.

VISIBLE MUTATIONS

Many abnormalities of eyes, wings, bristles and other parts of the body arose in the two treatment groups. These are loosely called "visible mutations," but actually

they belong to several different categories. Some are identical in appearance with well-known gene mutations in Drosophila. Others are somatic mutations, mosaics, and some may be the result of chromosome abnormalities such as deficiencies, translocations, etc. A large percentage of the flies showing these mutations were sterile. Such as are not sterile are now being bred in various combinations to determine the nature of the changes.

Table 5 gives the number of such mutations and their distribution among the three groups. Thirty-five occur in the T-4 group, fifteen in the T-2 and only three in the controls. This agrees in general with what Muller found in his experiments. The mutations grouped under "wing mutations" in the table include many different kinds of wing abnormalities, such as branched veins, one wing shorter than the other, notched and doubly notched, extended wings, depressed wings and several other forms of wing variation not previously seen in Drosophila.

Table 6 gives the mutation rate in the three groups, again using the word mutation loosely to include gene mutations, chromosome mutations and somatic mutations. Heavy treatment gave a mutation rate of .032; light treatment .006; while it falls to .00059 in the controls. This is of about the order of expectation and is in good agreement with Muller's results.

One point of considerable interest is that with the exception of the two mutations from Bar to full eye and

TABLE 5

A TABLE SHOWING THE KINDS AND DISTRIBUTION OF VISIBLE MUTATIONS APPEARING AS THE RESULT OF X-RADIATION OF THE MALE PARENT

	T-4	T-2	C
Bar to full eye	4	1	1
Wing mutations	17	13	1
Minute bristles	14	0	1
Red to white eye	0	1	0
Totals	35	15	3

TABLE 6

A TABLE SHOWING THE RATE OF MUTATION IN THE TWO TREATED GROUPS AND THE CONTROLS

	T–4	T–2	C
Total No. Flies	1077	2427	5007
No. mutations	35	15	3
Mutation rate	.032	.006	.00059

from red to white eye, all these mutations are dominants. The male alone was treated and these are the variations appearing in the F_1 generation, hence dominant. Probably many other mutations were induced by the rays, but being recessive in character could only be brought out by breeding the next generation.

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EXPERIMENTALLY-INDUCED METAMORPHOSIS IN ECHINUS

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(1) INTRODUCTION

IN a previous paper on dedifferentiation phenomena in sea-urchin larvae (Huxley, 1922), I suggested that dedifferentiation of larval tissues might be the determining factor in the normal metamorphosis of these forms. It is well known that the weight of the developing echinus rudiment eventually becomes so great that the organisms sink to the bottom. There I supposed that the conditions were not so favorable for the larval tissues as near the surface, where they are supplied with abundance of food and oxygen; that these larval tissues, which I had found very susceptible to adverse conditions and capable of a great degree of dedifferentiation, would then begin to dedifferentiate; and that, on this occurring, the less susceptible echinus rudiment would be more favorably situated as regards the "Kampf der Teile" within the organism, and would, therefore, not only not become dedifferentiated itself, but would grow at the expense of the regressing larval tissues. In other words, I supposed that the balance existing between larval and adult structures would be tilted in favor of the former. That such a balance between competing parts of an organic system can exist and may be tilted in one direction or the other by conditions is well known: and I had just come across a very striking example of it in Perophora (Huxley, 1921) where in good conditions of water, but without food, a zooid can maintain itself at the expense of an attached piece of stolon, which is gradually resorbed; whereas in unfavorable conditions of water, or when

very dilute poisons are added, the zooid begins to dedifferentiate and is then resorbed into the stolon, which may grow at the expense of the food material thus provided. (*Cf.* also similar effects in hydroids: Huxley and De Beer, 1923).

In the summer of 1926, thanks to the courtesy of Professor E. W. MacBride, F.R.S., I had the opportunity of testing my conclusions experimentally. I intended to continue the work this summer, but owing to various accidents the supply of plutei was not available. In the course of his work on the production of double hydrocoele in echinus, Professor MacBride rears large numbers of larvae of *Echinus miliaris* to metamorphosis in the tank-rooms at the Imperial College of Science. He very kindly put at my disposal a considerable number of specimens at various late stages of larval development. For this, and for advice on the problem, I should here like to tender my best thanks; without this source of supply, I do not suppose that I should have undertaken the work, since the business of rearing larvae to healthy metamorphosis is lengthy and laborious and demands a delicate technique.

The larvae at the Imperial College are reared in large plunger-jars with a supply of the diatom Nitzschia as food. Some specimens were left in the jars as controls. The rest were taken to King's College, where some were kept in small vessels holding one hundred to three hundred cc of sea-water, without circulation, and others were exposed to very dilute poisons. Some of these latter metamorphosed with startling rapidity, long before the controls, demonstrating the general correctness of my prophecy. In addition, some interesting dedifferentiation phenomena were observed.

In my previous paper I had established the fact that four- to five-day plutei were very rapidly dedifferentiated in $HgCl_2$ $\frac{m}{10^6}$, with arms absent or reduced to mere knobs after three days, and death in five days. In $\frac{m}{2 \times 10^6}$

the arms did not begin to be resorbed until two days, were not reduced to knobs or absence till six days; and death did not occur until seven days. I therefore chose $\frac{m}{2\times10^6}$ and $\frac{m}{3\times10^6}$ as the dilutions for the present work; of the two, the former appeared to be the most suitable.

(2) Precociously-induced Metamorphosis

The larvae were examined under the high power of the binocular dissecting microscope, and sorted out into various arbitrary classes according to the size of their echinus rudiment. The classification adopted was as follows. (1) Very late: echinus rudiment very large; signs of impending metamorphosis apparent; (2) late: echinus rudiment large; the larval arms usually pigmented near the tip, and often slightly shortened; (3) moderately late: arms still perfect; echinus rudiment somewhat smaller; (4) moderate: echinus rudiment well developed but considerably smaller than in (2)—about half the bulk; (5) early: echinus rudiment definite but small; (6) very early: echinus rudiment in earliest stages of development.

A number of specimens before treatment were sketched under the camera lucida to give the size of the larva and of the rudiment, and further sketches were made at intervals.

The first series of experiments were largely of a preliminary nature (see p. 369): the second series will here be described first.

Expt. 2. A culture of *Echinus miliaris* larvae was examined on June 28; all the *late* and *very late* classes (1 and 2) were rejected. The remainder were divided into two equal lots, and half left in the plunger-jar as "plunger control." The remainder were examined in detail, and some drawn, on 29/6. Some were placed for the night in a $\frac{m}{2{,}000{,}000}$ solution of $HgCl_2$ in sea-water, in which they remained for seventeen to eighteen hours; the rest

were left in sea-water. A number of specimens (some treated and some controls) were placed separately in 75 cc of fluid each. Two groups of four specimens of stage (3) and three specimens of stage (4) were placed in 180 cc of $HgCl_2$ solution, and corresponding groups each in 180 cc of sea-water.

TABLE I

EFFECTS ON METAMORPHOSIS OF TREATMENT WITH DILUTE $HgCl_2$ SOLUTION

			Treatment Result: After ¾–1 day					After 2½ days				
			dead	full larval	larval, but arms slightly resorbed	early to middle metamorphosis	late to complete metamorphosis	dead	full larval	larval, but arms slightly resorbed	early to middle metamorphosis	late to complete metamorphosis
Stage	Expt. 2											
3	5											
4	6											
5	1	$HgCl_2$										
	12		2		2	2	6					
3	5											
4	5	non-plunger										
5	1	control										
	11		.....	4	6	.....	1					
3	.....							.....	.....			
4	?	plunger						.....	17			
5	?	control						.....	4			
	25							.....	21	3	.....	1
Stage	Expt. 2a											
1	1											
2–4	9											
5	2	$HgCl_2$										
	12		1	.....	.....	.....	11					

Expt. 2. $HgCl_2 \frac{m}{2 \times 10^6}$ normal; non-plunger control in sea-water in small vessels; plunger control in plunger jars.

Expt. 2a. $HgCl_2 \frac{m}{2.6 \times 10^6}$ normal. Specimens taken from plunger control of Expt. 2.

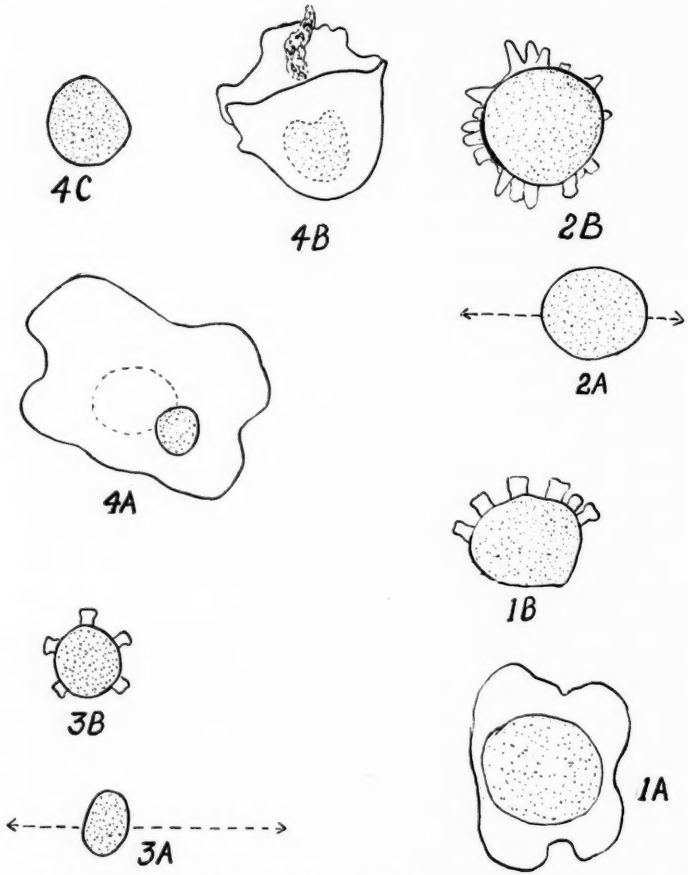
On July 7, the plunger controls were examined, and a number of those which had remained fully larval were placed in a $\frac{m}{2{,}000{,}000}$ solution of $HgCl_2$. These constituted Expt. 2a.

The results of these two experiments may be summarized as follows (Table I). The result is obvious. In Expt. 2, 80 per cent. and in Expt. 2a, 100 per cent. of the surviving specimens (90 per cent. for both experiments taken together) had completely or partly metamorphosed after eighteen to twenty-two hours in the $HgCl_2$ solution. If we reckon the percentage on the total number put in instead of the survivors, it is 67 per cent. (Expt. 2); 92 per cent. (Expt. 2a), and 71 per cent. (both Expts.). Meanwhile, in the controls (Expt. 2, plunger and non-plunger together) 5.6 per cent. had metamorphosed, the plunger controls only being examined after two and one half days instead of one day. No treated specimens remained fully larval after twenty-two hours; while 69 per cent. of the controls did so.

The mortality was nil in the controls, 12.5 per cent. in the treated specimens (this figure includes two specimens which though not dead were obviously badly damaged). An important fact is that some of the artificially metamorphosed echini were far smaller (less than 20 per cent. of the volume) than normal: *e.g.*, compare Fig. 3B with Fig. 1B. Such small echini were of course produced from larvae with less advanced echinus rudiments. (*Cf.* production of miniature froglets by thyroid.)

Some of the remainder of the plunger control of Expt. 2 were taken on July 2 as non-plunger controls to Expt. 2a; two were in stage (1); even these did not reach middle-to-late metamorphosis until six days, and practically complete metamorphosis after seven days. After eight days oedematous degeneration set in.

A specimen on the verge of metamorphosis, with very large echinus rudiment and incipient arm-resorption (stage 1), kept as finger-bowl control, only metamorphosed after more than five days.



All figures are sketched, with the aid of the *camera lucida*, to the same scale (except Fig. 11).

FIG. 1A. Expt. 1, No. 3 (stage 2–3). Before treatment; outline from below to show relative size of echinus rudiment.

FIG. 1B. The same specimen (seven days later). Complete metamorphosis after $HgCl_2$ treatment.

FIG. 2A. Expt. 1, No. 4 (stage 3–4). Echinus rudiment and maximum width before treatment.

FIG. 2B. The same specimen (seven days later). Complete metamorphosis after $HgCl_2$ treatment. Tube feet active; specimen beginning to show oedema.

One of the non-plunger controls of Expt. 2 appeared to have a somewhat dedifferentiated echinus rudiment when the experiment was started. This specimen showed progressive dedifferentiation of the larva, with complete dedifferentiation of the echinus rudiment. No tube-feet were formed. After twelve days it had become a spheroidal mass similar to those obtained by me by treatment

Expt. 1.

$HgCl_2$ treatment for 17 hours; then sea-water; results after 6 days

	Total	Dead	Dedifferentiated larvae	Partial metamorphosis	Complete metamorphosis
$HgCl_2$ treated	8	1	3	1	3
Control (finger-bowls)	9	.....	7	2	.....

of early larvae before the development of the echinus rudiment (Huxley, 1922). (*Cf.* Fig. 8, which figures a similar specimen, whose career, however, was not followed in such detail.

The earlier experiment (Expt. 1) with $HgCl_2$ is summarized above. $\frac{m}{2\times10^6}$ and $\frac{m}{3\times10^6}$ $HgCl_2$ solutions were employed, both for seventeen hours; no significant difference was observed between their effects, so the results are lumped together. The animals were not examined in detail at once after removal from the solutions,

FIG. 3A. Expt. 1, No. 6 (stage 4–5). Echinus rudiment and maximum width before treatment.

FIG. 3B. The same specimen (seven days later). Complete metamorphosis after treatment with $HgCl_2$. Note small (but increased) size of echinus.

FIG. 4A. Expt. 1, No. 8 (stage 5–6). Outline (arms omitted) to show relative size of echinus rudiment before treatment.

FIG. 4B. The same specimen six days later, after treatment with $HgCl_2$. Marked larval dedifferentiation; arms reduced to stumps; debris extruded from mouth. The dark body in the center is the stomach. The echinus rudiment is not figured; it is only slightly larger than in 4A.

FIG. 4C. The same specimen two days later than in 4B. Abnormal metamorphosis. All trace of larval tissues resorbed. Echinus rudiment increased in size, but without trace of tube-feet or spines. Slowly rotating.

and the fuller notes were taken six days from the beginning of the experiment. Animals from stages 2 to 5 were taken, with corresponding stages in the controls. In the $HgCl_2$ series all the animals in stages 2 and 3 metamorphosed, and one of two in stage 4. The other stage 4 and both stage 5 specimens showed dedifferentiation of the larva without development of the echinus rudiment. This is parallel to the results obtained by Muller (1913, 1914) on hydroids: the younger the bud-rudiment, the more readily was it resorbed by the piece of stem to which it was attached.

The partial metamorphosis of two specimens in the treated series was peculiar, in that, though the larval tissues were completely resorbed, the echinus rudiment was also dedifferentiated, in regard at least to losing its tube feet. The metamorphosis was also unusually long delayed, but when it came, it came fairly rapidly, *i.e.*, the balance between the two components was not so quickly or so thoroughly tilted, and as a result there was a more balanced competition in unfavorable circumstances, which led to the dedifferentiation of both components, though with incomplete victory of the echinus rudiment (Figs. 4C, 5C). Somewhat similar results were also found in a few controls (after much longer periods), but here the resorption of the larval tissues was not quite complete.

The majority of the specimens which metamorphosed as the result of treatment (in all experiments) died within the next two to four days. That this is not a necessary result of the treatment, however, is shown by the fact that one specimen from Expt. 2a was alive and healthy, in spite of the absence of suitable food, eight days after treatment. It is, I think, clear that by varying the strength of the solution and the length of exposure, $HgCl_2$-metamorphosed specimens could be obtained which were perfectly normal and viable.

(3) Dedifferentiation

Some peculiar dedifferentiation phenomena were observed, notably among the untreated finger-bowl controls.

Fig. 7 shows a most peculiar condition, in which one larval arm remained unaffected long after all the rest were completely resorbed. This state of affairs lasted for over a week.

Fig. 8 shows the extreme of dedifferentiation (*cf.* some of the figures in my earlier paper). All trace of arms, ciliated band, mouth, etc., has disappeared, and extreme size-reduction has occurred. The irregular outline and persistence of cavities within distinguishes this from the results of resorption of larval tissues into the echinus rudiment.

Fig. 9 is moderate larval dedifferentiation, accompanied by slight dedifferentiation of the echinus rudiment, as shown by its hazy outline. Fig. 10 shows a stage intermediate between Fig. 9 and Fig. 8. Fig. 12 represents a type of dedifferentiation not infrequent in these cultures, in which the shrinkage in size which usually accompanies dedifferentiation is prevented by oedema. All trace of arms, preoral lobes, etc., has disappeared; a partially resorbed spicule is still visible, and two pedicellariae are preserved. Some similar, but not so extreme cases were obtained with younger specimens (*e.g.*, Fig. 12a and b of my previous paper).

Fig. 11 illustrates a very peculiar condition observed only once. A metamorphosed echinus had died during the night. On its spicules were cell-masses, as figured, which undoubtedly seem to have been produced by the outwandering of cells (? mesenchyme cells) from the dying organism to more favorable environment. They survived a couple of days, and in some respects recall the cell-masses produced by the artificial dissociation of sponges and coelenterates. A clumping of mesenchyme cells during larval dedifferentiation was observed by me previously (see Fig. 2b of my previous paper).

(4) Discussion

Professor MacBride has objected to me that the normal metamorphosis is of explosive rapidity, and need not

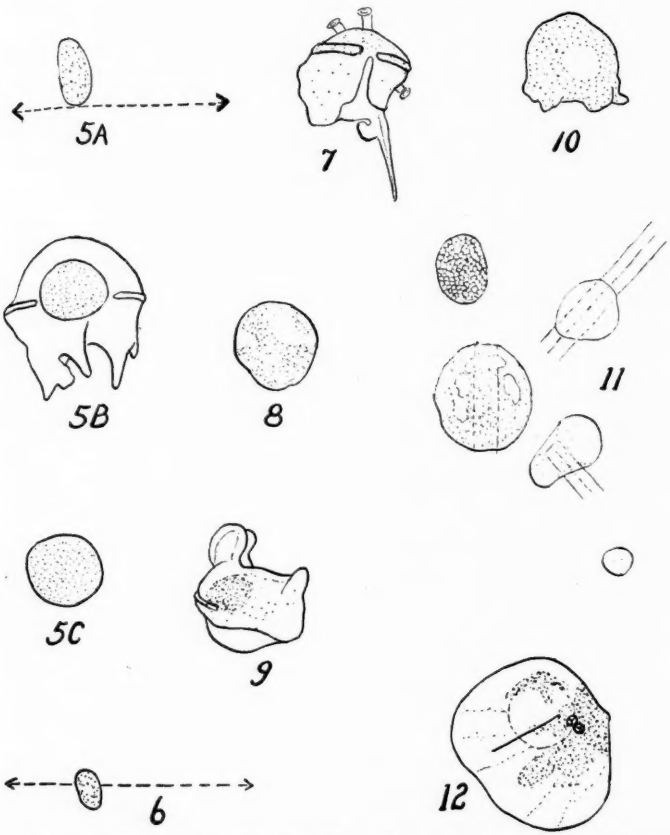


FIG. 5A. Expt. 1, No. 2 (stage 4). Echinus rudiment and maximum width before treatment.

FIG. 5B. The same specimen seven days later, after treatment with $HgCl_2$. Moderate larval dedifferentiation.

FIG. 5C. The same specimen two days later. Abnormal metamorphosis as in Fig. 4C. Death supervened next day.

FIG. 6. Expt. 1, No. 5 (stage 6). Before treatment with $HgCl_2$, to show size of echinus rudiment and maximum width. Treatment caused larval dedifferentiation together with reduction of the echinus rudiment, and no metamorphosis occurred.

FIG. 7. Expt. 1. Untreated control (originally stage 3) after thirty-two days in a finger bowl. Marked larval dedifferentiation except of one arm; ciliary band still moderately active. No sign of dedifferentiation in echinus rudiment: tube-feet active.

be preceded by resorption of arm-tips. With regard to the former point, it should be noted that in nearly all cases of complete or nearly complete metamorphosis after $HgCl_2$ treatment, the result was already a *fait accompli* on examination the morning after the animals had been placed in the solutions. Metamorphosis is thus certainly rapid; as soon as new material is available, it is proposed to watch continuously the sequence of events in the treated specimens and to see whether here, too, the actual metamorphosis may not be explosive, as I anticipate it will be.

On the other hand, it is bound to be less explosive and less rapid the smaller is the echinus rudiment, on simple principles of biological mass-action (see Huxley, 1921, on Perophora). Where in addition the echinus rudiment is young and itself *affected* by the toxic solution, we should anticipate a much closer physiological balance between it and the larval tissues; and since in addition it is small, it will be harder for the resorbed tissues of the larval

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FIG. 8. Expt. 1. An untreated control after twenty-four days in a finger bowl. Extreme larval dedifferentiation. There are still clear cavities within, and the outline is slightly irregular, which distinguishes this from the abnormal metamorphosis of Figs. 4 and 5.

FIG. 9. Expt. 1, No. 10 (stage 4 before treatment). Twenty-four days after treatment with $HgCl_2$. Failure to metamorphose. Marked larval dedifferentiation; echinus rudiment slightly dedifferentiated.

FIG. 10. Expt. 1, No. 5 (stage 6 before treatment). Thirty-seven days after treatment with $HgCl_2$. Failure to metamorphose. Extreme larval dedifferentiation. Opacity too great to detect if echinus rudiment present. Still rotating. Note the great size-reduction, the stomach still visible as a clear space, and the vestiges of arms.

FIG. 11. Higher power sketches (*camera lucida*) of cell masses which remained alive and migrated out from one dying metamorphosed echinus eight days after treatment with $HgCl_2$. Most of them had migrated out along spines, as indicated. In one the cell boundaries are indicated to scale. One shows irregular spaces within. The smallest mass found has been sketched to scale.

FIG. 12. Expt. 1. Untreated control. Failure to metamorphose after twenty-six days, though in stage 2 at beginning of experiment. Oedematous type of larval dedifferentiation. Complete resorption of arms; body swollen. Part of a spicule still not resorbed; two pedicellariae visible. Echinus rudiment still present, but dedifferentiated.

rudiment all to be forced into it. For both reasons a slow and possibly incomplete metamorphosis is to be anticipated. This seems occasionally to occur (see above, p. 370).

With regard to the second point, I do not think it of great importance. Once the larvae sink away from the surface, they are exposed to less favorable conditions. Before metamorphosis, these may or may not have become visibly expressed, the first sign being the arm-tip dedifferentiation, before the physiological balance between echinus rudiment and larval tissues is tilted. This balance is essentially one of food competition. In the absence of the surface diatoms, each component is starved, and seeks to draw on the other; once a certain point is reached, the process runs rapidly to its conclusion. The effect of artificial dedifferentiation of the larva by toxic solutions is to weaken the larval tissues and so enable the less-affected echinus rudiment to draw on them before it otherwise would be able to do so.

Precisely similar results, of incomplete and atypical metamorphosis, may be observed after artificial metamorphosis of anuran tadpoles by means of thyroid. If too strong doses are employed, the animals almost invariably die during metamorphosis. The probability of death is increased the younger the animals are when treated. Further, in young larvae, any large or moderate dose of thyroid causes disharmonic metamorphosis, with limbs not nearly large enough for adult life. This is due to elementary facts concerning the rate of limb development, and the different threshold of susceptibility to thyroid of limbs and tail (Huxley, 1923). It is only by very careful dosage, and preferably also by the aid of special measures, such as keeping in an oxygen-rich atmosphere, that thyroid-treated tadpoles live through the metamorphosis. None the less, no one now hesitates to believe that the thyroid hormone is the normal cause of amphibian metamorphosis. Facts such as these seem

a further support against scepticism; it seems as unlikely that in normal metamorphosis of echinus some mechanism wholly alien to this dedifferentiation and resorption of larval tissues is at work, as to suppose that the mechanism of artificial thyroid-induced amphibian metamorphosis is not essentially similar to the normal. In any case, the criticisms of Pérez (1923), to the effect that since dedifferentiation is always a pathological phenomenon, it therefore can not possibly have anything to do with a normal process like metamorphosis, is seen to be ill-founded.

Summary

(1) Treatment of advanced larvae of *Echinus miliaris* with very dilute solutions of $HgCl_2$ (about $\frac{m}{2\times10^6}$ rapidly brings about precocious metamorphosis, a young echinus in one case surviving eight days in spite of the absence of food.

(2) This appears to be caused through the differential susceptibility of larval tissues and echinus rudiment. The former are more affected by the poison, begin to dedifferentiate, and can then be readily resorbed by the echinus rudiment.

(3) When the echinus rudiment is small, metamorphosis is less rapid and may be incomplete, both larval and echinus tissues being dedifferentiated. Even when metamorphosis in such cases is complete, it is often abnormal, the young echinus possessing small or no tube-feet or spines. Parallel abnormalities are to be found in artificial (thyroid-induced) metamorphosis in anuran tadpoles.

(4) It is suggested that a similar mechanism is operative in the normal metamorphosis of echinids, the larval tissues dedifferentiating when the weight of the echinus rudiment causes the organism to sink away from the favorable food and oxygen conditions of the surface.

(5) Some dedifferentiation phases are figured.

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SHORTER ARTICLES AND DISCUSSION

A PECULIAR REGENERATION OF THE FIRST ABDOMINAL APPENDAGE IN THE MALE CRAYFISH CAMBARUS VIRILIS HAGEN[1]

SEVERAL years ago the writer obtained a number of specimens of the crayfish *Cambarus virilis* Hagen in Wabinosh Bay in the northwest part of Lake Nipigon in northern Ontario, Canada. Among these was one male specimen which exhibited a very peculiar modification of one of the first abdominal appendages. Since no record of the regeneration of any similar structure could be found, a description of it is given below.

In the normal representative of this species the first abdominal appendages of the male are modified in structure to form a pair of slender, styliform copulatory organs with slightly recurved tips as shown in the figure (a). The thoracic appendages, on the other hand, are strong, jointed, uniramous appendages as shown in the figure (c) and these are used in walking. In the present specimen the first abdominal appendage of the right side was perfectly normal in structure but that of the left greatly resembled the last thoracic leg. Its basal portion, however, was similar to normal but the terminal part consisted of a number of separate joints which not only resembled those of the thoracic leg in shape but were also provided with a similar armature of hairs and spines, figure (b).

Steele (1904) reports the results of actual experimental work on the regeneration of the first abdominal appendages of the male of this species of crayfish, apparently with the production of parts similar to the original. In general the parts produced by regeneration resemble the normal structure more or less but many abnormalities undoubtedly do occur. It also sometimes happens that structures of a less highly evolved type replace the original. Thus Emmel (1906), in discussing the regenerative process in the cheliped of the lobster, points out that "in the regeneration process of the cheliped we meet with recapitulation of characteristic phases of its ontogeny."

Hence, the chief interest of the regenerated appendage which has been described arises in connection with its relation to the

[1] Contribution No. 316 from the Zoological Laboratory of the University of Illinois.

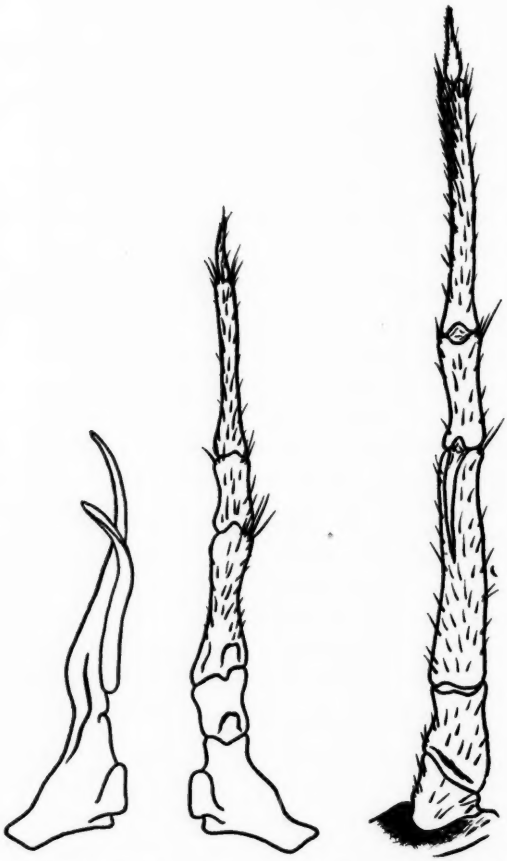

FIG. A. Typical first abdominal appendage of male (right side).
FIG. B. Regenerated first abdominal appendage resembling thoracic leg.
FIG. C. Typical fifth thoracic leg.
(Figures read from left to right.)

fact that the appendages of the crayfish are thought to be homologous and that the various types of legs which are normally present will, therefore, represent forms which have been evolved from a common and supposedly simpler type—namely, the typical biramous appendage of the abdominal segments. In support of this contention it is common to cite the case of the development of the walking legs of the lobster. This crustacean

passes through a series of free-living larval stages before coming to resemble the adult and in some of these earlier stages the thoracic legs are typical biramous appendages. Accepting this as the correct explanation of the relation of the appendages, then, if one may be allowed to theorize on the basis of such a limited amount of material evidence as is offered by the single specimen under discussion, it is apparent that the present case would represent an instance in which the evolutionary process had been re-awakened later in life in some unknown manner with the result that a more highly evolved appendage has been formed during regeneration.

In connection with the examination of this specimen, a dissection of the internal organs was also made. This revealed the fact that the anterior lobe of the testis on the left side (that is, the same side as the regenerated leg) was misshapen and shrivelled, and that its surface was covered with large numbers of small particles which proved to be the spores or cysts presumably of some parasite. Sectioning of the testis also showed unmistakable evidence of the causes which led to the shrivelling. Although the anterior end of the lobe was apparently quite normal in histological structure and even showed mitotic figures indicative of active cell division, the posterior end, on the contrary, showed signs of extensive degeneration. Here the germ cells were reduced to unrecognizable masses or were even entirely eliminated and the containing tubules shrivelled up or disintegrated. The areas between the tubules were invaded in places by an extensive proliferation of small cells—evidently a pathological condition. Some of the spores or cysts were also present embedded in the degenerate tissues. As to the identity of the invading organism, the question is not yet settled, and in view of the fact that it is not desired to suggest any definite connection between the two conditions which have been described, that question can well be left in abeyance. In spite of this, the coincidence is a very remarkable one and will furnish the starting point for further investigation.

F. B. ADAMSTONE

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POLYGAMY IN THE BIRDS OF PARADISE

Despite the attraction they have exercised on naturalist and the general public alike, almost nothing is known of the habits of the birds of paradise in their wild state. The courtship habit of the males assembling on certain trees for the purpose of display has been described by Wallace (19) and other writers, and the display itself has been the subject of several papers, the latest accounts being those of Ogilvie-Grant, (12) for *Paradisea minor;* Ingram, (8) for *Cincunnurus regius;* and Seth-Smith, (16) for *Diphyllodes magnifica.* On the subject of polygamy, however, we are still in complete ignorance. For long it has been tacitly assumed that the Paradiseidae are polygamous. Lesson first made this assertion, and although Wallace pointed out the lack of evidence, the idea now seems to be firmly fixed. (4) Thus Goodfellow(6) refers to *Paradisea apoda* as polygamous, while Seth-Smith (16) states that "all, as far as is known, are polygamous." In neither case is any evidence advanced. Sharpe, (17) Elliot, (5) and Gould (7) make no reference to the subject. Sharpe, it is true, mentions that males appear to preponderate in *Paradisea minor;* on the other hand, he states that both sexes of *Diphyllodes magnifica* dance; and that *Phonygamma gouldi* leave the dancing place in pairs, it being very rare to see a male accompanied by more than one female. D'Albertis, writing of *Paradisea raggiana,* states that the females were, at the time of his visit to New Guinea, less abundant than the males, as he believed it to be the season of incubation; but adds that he has no information on the nidification.

In the absence of direct evidence, it appears very rash to make any definite assertion as to the habits of the birds on this point; but in view of the great revival of interest in bird behavior recently, it might prove of some interest to weigh carefully the probabilities for and against the proposition.

Perhaps the most weighty argument in favor of the polygamy of the birds of paradise is the extraordinary development of the secondary sexual characters in the male. Professor Julian Huxley told the writer that he thought such a state unlikely without polygamy, and similar ideas seem to have influenced others on this point. Nevertheless, considerable differences occur between the sexes in many ducks, and in the hen harrier (*Circus cyaneus*), all of which are monogamous. Beebe (2) has come

to the conclusion that most of the pheasants are not invariably polygamous, this state depending on the number of hens; yet these birds show very great sexual dimorphism.

Another argument in favor of polygamy is the elaborate courtship antics of the males. But, as mentioned above, in *Diphyllodes magnifica* both sexes are said to display; while on the other hand, in several of the ducks, the male alone shows off. (14) Again, Darwin (4) says that a Scandinavian ptarmigan, which is monogamous, actually holds *leks* similar to those of the blackcock. Another argument in favor of polygamy is the frequent separation of the females and young males from the adult males at all times except during the breeding season.

As I have pointed out elsewhere, (20) practically all polygamous birds nest on the ground. This is very possibly mere coincidence, since most of the polygamous birds belong to the groups of the game-birds, the waders and the Ratites, in all of which groups ground-nesting is a characteristic, whatever the number of mates. However it might be worth while considering the bearings of this on the point under discussion. The nests of the following birds of paradise have been described:—*Paradisea augusta-victoria,* (11) *Ptilorhis rudolphi,* (18) *Pt. victoria,* (9) *Pt. paradisea,* (3) *Craspedophora alberti,* (10) *Drepanornis cervinicauda,* (7 and 17) and *Manucodia jobiensis.* (13) In each case the nest was found to be a fairly substantial structure of twigs or similar materials, and was placed in a tree or bush. That of *Pt. paradisea* was actually decorated with shed snakes' skins. In this case the hen was sitting when the nest was discovered and a cock bird had just been shot near-by—a point which may or may not have any significance. The only other information available of the nidification of the group consists in two native tales. One of these, quoted by Wallace, (19) is to the effect that *Epimachus speciosus* lays its eggs in holes underground or under rocks, these having two apertures, so that the bird can enter by one and leave by the other. The second tale, related by Macgillivray, has been questioned by Sharpe (17) and Le Souef. (10) It is that *Craspidornis magnifica,* unlike all the other rifle birds whose nidification is known, lays several eggs in a hollow tree.

Summary

(1) There is no direct evidence in favor of the traditional assumption that the birds of paradise are polygamous.

(2) In favor of the theory of polygamy: the marked secondary sexual characters of the males; the elaborate courtship; and the frequent separation of the sexes.

(3) Against the theory of polygamy: the reported mutual courtship of *Diphyllodes magnifica;* the substantial and arboreally-placed nests; the pairing of *Phonygamma gouldi;* and the possible co-operation of the male of *Ptilorhis paradisea* in guarding the nest (cf. the male mallard, *Anas platyrhyncha*).

Conclusions. While it is quite possible that some forms, *e.g., Paradisea apoda,* will prove to be polygamous, it is not likely that all species are so; in particular, *Phonygamma gouldi* and *Diphyllodes magnifica* will probably be found to be monogamous. It is highly desirable that future expeditions should devote more attention to field notes of the birds and less to mere collecting of skins. Perhaps the best chance of solving the problem, so far as *P. apoda* is concerned, lies in the study of the birds liberated in Little Tobago, which is more readily accessible than New Guinea, and where good work has already been done (1).

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THE PRIMITIVE TYPE OF ARACHNID APPENDAGE

MANY of the older views in regard to the phylogeny and relationships of the major arthropod groups have in recent years undergone modification or abandonment. The time-honored belief that the extinct trilobites occupied a position near that of the forebears of both the Crustacea and the Arachnida has been generally discarded, following the discovery of fossil forms showing a pair of antennae and body appendages with a reduced number of segments, as well as other structures quite characteristic of living crustaceans.

The old theory that insects are the descendants of types similar to annelid worms has been dropped by many for the crustacean theory of the origin of Insecta. This has been due particularly to the evidence presented by the study of ancient types of fossil insects, the further study of apterygote insects and the discovery of the primitive hexapod group Protura, related to the crustacea-like Pauropoda on the one hand and the collembolan insects on the other.

The acceptance of the crustacean theory of insect origin involves a different perspective in working out the homology of the appendages of Insecta. Contributing greatly to this task is the theory that the pleural plates of insects are derived from the basal segment of the leg of insect ancestors; in other words, that an additional leg segment should be recognized in insects. This segment has been termed the subcoxa.

Hansen[1] has claimed that in the Crustacea three, not two, segments should be recognized in the undivided part of a generalized (primitive) crustacean limb. He has further suggested[2] that

[1] Hansen, H. J., "Zur Morphologie der Gliedmassen und Mundtheile bei Crustacean und Insecten." *Zool. Anz.*, Bd. XVI, pp. 193–198 and 201–212 (1893).

[2] Hansen, H. J., "Studies on Arthropoda," II, 176 pp., 8 pls. Copenhagen. Gyldendalske Boghandel.

there is a strong probability that the primitive crustacean limb was uniramous instead of biramous.

While working on the segmentation and musculature of the legs of apterygotan insects and various primitive arthropod groups the writer has been forced to the conclusion that the generalized arachnid leg not only is uniramous, but that it probably includes one more segment than Hansen allows for the Crustacea. In such groups of living arachnids as the Pycnogonida, Solpugida, Cyphophthalmi, Ricinulei and the primitive Acarina of the family Labidostommatidae, eight complete and functional leg segments are evident in addition to the claws, now commonly regarded also as representing a segment, the pretarsus.

This generalized arachnid leg may be described as follows: The more proximal segments are articulated by means of a double hinge (dicondylic) and each is moved by a levator and a depressor muscle which originates in the segment adjacent (proximally) to the one they move. The more distal segments, usually those beyond the bend of the leg, are articulated by means of a single dorsal hinge and each is moved by a flexor muscle only, which also originates in the adjacent proximal segment. The pretarsus is moved by a pair of muscles, an extensor which originates in the adjacent segment proximally, and a flexor which originates from the two segments proximally. The following names are suggested for the nine segments of a generalized arachnid leg: Subcoxa, coxa, coxal trochanter, femoral trochanter, femur, patella, tibia, tarsus and pretarsus. The suggestion is here made that this type of a leg is the most primitive that has yet been demonstrated for any class of true arthropods, and for the time being should be regarded as a generalized type for the Arthropoda.

Trilobites certainly are crustaceans, and *Limulus* is to be regarded as being far from primitive, although it does combine in a striking manner certain arachnid and crustacean characters. The segmentation and musculature of the legs of *Limulus* are of a highly modified and atypical arachnid type.

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